Repellent Efficacy of Monotypic and Binary Formulations of Psidium Guajava and Basella Rubra Leaf Extracts against Aedes aegypti

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ABSTRACT

Background: The reliance on synthetic mosquito repellents like DEET necessitates the exploration of safer botanical alternatives. This study evaluated the spatial repellency of Psidium guajava (guava) and Basella rubra (Malabar spinach) leaf extracts, both monotypic and binary, against adult female Aedes aegypti mosquitoes.

Methods: Using a custom Excito-Repellency Test Chamber Assay, seven formulations were tested: 100% P. guajava, 100% B. rubra, three volumetric combinations (50:50, 25:75, 75:25), a 12% N,N-diethyl-meta-toluamide (DEET) positive control, and an untreated negative control. Fifteen non-blood-fed mosquitoes were exposed per trial, with chamber escape rates tracked over 15 minutes. Relative efficacy was analyzed using One-Way ANOVA and Tukey's HSD post-hoc test.

Results: Monotypic Psidium guajava extract achieved 66.67% mean repellency, demonstrating performance statistically comparable to DEET (86.67%, $p = .1936$). In contrast, monotypic Basella rubra exhibited poor vapor-phase activity at 53.33% repellency, falling significantly below the positive control ($p = .0249$). Binary blends across all volumetric ratios produced a uniform 62.22% repellency rate. Rather than demonstrating synergy, these combinations reflected an additive effect, where incorporating as little as 25% P. guajava supplied enough volatile terpenoids to elevate the spatial performance of the B. rubra base to statistical equivalence with DEET.

Conclusion: P. guajava leaf extract is a highly effective monotypic botanical spatial repellent. While pure B. rubra lacks the volatility required for vapor-phase deterrence, combining it with guava extract creates a stable, viable alternative formulation for vector control.

Keywords: Additive effect, Aedes aegypti, Basella rubra, Excito-repellency, Psidium guajava.

INTRODUCTION

Dengue fever continues to pose a major public health challenge worldwide, with recent estimates showing over 100 million symptomatic cases each year across more than 125 countries (Clarke et al., 2024). The Philippines is particularly vulnerable to these outbreaks. Recent data from the Department of Health points to a steady rise in infections, driven largely by rapid urbanization and shifting climate patterns (DOH, 2024). As case numbers climb, it has become clear that conventional mosquito control strategies are falling short. For decades, vector management has depended heavily on synthetic insecticides and personal repellents. However, prolonged exposure has led to widespread insecticide resistance in local Aedes aegypti populations, making standard chemical interventions increasingly unreliable (Malijan et al., 2024). Beyond resistance, the environmental toxicity and potential health risks associated with synthetic repellents like N,N-diethyl-meta-toluamide (DEET) have prompted researchers to look for safer, more sustainable alternatives.

Plant-based repellents offer a practical path forward. Because they are biodegradable and generally have lower human toxicity, they carry a much lower risk of triggering resistance (Lopez et al., 2025). Many botanicals contain volatile bioactive compounds that actively interfere with mosquito host-seeking behavior (Wood et al., 2024). Psidium guajava (guava) and Basella rubra (Malabar spinach) are two accessible plants in tropical regions with known phytochemical profiles rich in these active metabolites. While researchers have documented the individual biological properties of both plants, their combined repellent efficacy against A. aegypti remains unexplored. Combining botanical extracts often produces synergistic effects, which can significantly extend protection time and boost overall repellency to levels comparable to synthetic products (Lopez et al., 2025).

To address this, our study evaluated the mosquito-repellent properties of P. guajava and B. rubra leaf extracts against adult A. aegypti, testing them both individually and as a combined formulation. Through standardized laboratory bioassays, we compared their effectiveness and protection duration directly against DEET. The primary goal was to determine whether this specific botanical combination can yield a natural, cost-effective, and scalable repellent, providing a viable alternative for communities heavily impacted by dengue.

Synthesis of Related Studies

As mosquito-borne diseases continue to spread, researchers are increasingly looking toward plant-based repellents to replace synthetic chemicals like DEET. The push for these natural alternatives is largely driven by real public health hurdles — specifically, the environmental toxicity of chemical sprays and the fact that mosquito populations, especially Aedes aegypti, are rapidly developing resistance to them (Malijan et al., 2024; Rao et al., 2022). Because botanical extracts are naturally packed with secondary metabolites, they offer a practical way to manage mosquito vectors without the ecological baggage of conventional insecticides.

Guava (Psidium guajava) is emerging as a particularly strong candidate in this area. Recent work shows that guava leaf extracts are highly effective at killing Aedes aegypti larvae, with mortality rates scaling directly with the concentration of the extract (Kartini & Sofia, 2022). This larvicidal power comes from a complex mix of bioactive components—including quercetin, catechin, and tannins—which trigger fatal oxidative stress in the larvae (Aremu et al., 2024). But the plant does more than just kill larvae. The volatile oils found in P. guajava leaves actually prompt strong avoidance and excito-repellent behaviors in adult mosquitoes (Jhaiaun et al., 2021). On top of vector control, specific compounds like gallic acid and catechin extracted from the plant's leaves and bark even show direct antiviral activity, successfully inhibiting the replication of the dengue virus (DENV-2) in laboratory settings (Trujillo-Correa et al., 2019).

Basella rubra, commonly known as Malabar spinach, is another plant showing significant, though underexplored, potential. Chemical profiling reveals a dense array of pharmacologically active compounds in the plant, particularly betacyanins, flavonoids, saponins, and terpenoids (Chaurasiya et al., 2021; Krishnappa & Elumalai, 2013). While it is mostly recognized in traditional medicine for its strong antioxidant, antimicrobial, and anti-inflammatory benefits (Reddy et al., 2023), the specific terpenoids and flavonoids it contains are structurally very similar to compounds known for insecticidal and spatial repellent activity. This makes B. rubra a promising agent for vector management.

Research Gap

Looking at the current literature on botanical vector control, the biggest blind spot is that no one has actually tested Psidium guajava and Basella rubra together against Aedes aegypti. Every study so far treats these plants in total isolation. Single-plant extracts, however, rarely match synthetic repellents like DEET because their spectrum of active compounds is fairly narrow, which usually leads to quick evaporation and weak spatial performance. Blending the two extracts opens up the possibility of phytochemical synergy—letting guava's catechins and tannins work alongside the flavonoids and terpenoids in Malabar spinach to create a stronger, longer-lasting repellent effect than either plant could achieve on its own. Right now, there are no standardized protocols for choosing the best extraction solvents, nor is there data on what mixing ratios (such as 1:1 or 2:1) maximize repellency without causing chemical interference between active compounds.

There is also a clear imbalance in how thoroughly each plant has been investigated. While P. guajava has solid empirical backing for killing larvae, repelling adult mosquitoes, and suppressing dengue virus replication, B. rubra is backed mostly by theory. Most research on Malabar spinach focuses on its nutritional benefits, antioxidant profile, or general antimicrobial properties. Its insecticidal potential is usually inferred from its chemical makeup rather than proven through direct bioassays on mosquitoes. Without baseline data establishing its effective concentration thresholds or protection windows against adult A. aegypti, its actual usefulness in vector management remains unverified.

METHODOLOGY RESEARCH DESIGN

This study utilized a quantitative, true experimental laboratory design to evaluate and compare the mosquito-repellent efficacy of Psidium guajava (guava) and Basella rubra (Malabar spinach) leaf extracts against adult female Aedes aegypti mosquitoes. Testing was conducted under controlled environmental conditions, measuring spatial repellency and contact irritancy through an Excito-Repellency Test Chamber Assay.

Plant Material Collection and Extraction Procedure

Fresh, undamaged leaves of P. guajava and B. rubra were collected from disease-free plants. The leaves were washed thoroughly with distilled water to remove surface debris and shade-dried at room temperature (28°C) until reaching a constant mass. The dried leaves were pulverized into a fine powder using a mechanical grinder.

For crude extraction, the powdered materials were subjected to cold maceration using 95% ethanol as the solvent at a 1:5 (w/v) plant-to-solvent ratio for 24 hours with periodic agitation. The resulting mixtures were initially filtered through sterile cheesecloth, followed by filtration through Whatman No. 1 filter paper. The filtrate was subsequently concentrated under reduced pressure using a rotary evaporator set at 40°C to remove solvent residues. The resulting crude sticky extracts were stored in airtight, light-resistant amber glass vials at 4°C until formulation preparation.

Experimental Treatment Groups and Controls

A total of seven experimental treatment groups were prepared to evaluate single-plant extract performance, synergistic blend ratios, and established benchmarks. The formulations were prepared as follows:

1. Treatment 1: Pure Psidium guajava extract.
2. Treatment 2: Pure Basella rubra extract.
3. Treatment 3: Combined extract formulation at a 1:1 volumetric ratio.
4. Treatment 4: Combined extract formulation at a 2:1 ratio (P. guajava to B. rubra).
5. Treatment 5: Combined extract formulation at a 1:2 ratio (P. guajava to B. rubra).
6. Positive Control: Commercial N, N-diethyl-3-methylbenzamide (DEET, 12% v/v).
7. Negative Control: Untreated filter paper (impregnated with solvent alone and fully dried). Each treatment solution was uniformly applied to Whatman No. 1 filter papers (12x15 cm) at a fixed volume of 1.5 mL using a micropipette. Treated papers were allowed to air-dry under a laminar flow hood for 30 minutes prior to bioassay testing to ensure complete solvent evaporation.

Test Organisms and Maintenance

Pathogen-free, 5 to 7-day-old non-blood-fed adult female Aedes aegypti mosquitoes were obtained from a standardized colony maintained in an insectary facility. Environmental parameters inside the insectary were held constant at 27°C, 75% relative humidity, and a 12:12-hour light-dark photoperiod. Prior to the assay, test mosquitoes were starved of 10% sucrose solution for 4 hours to equalize activity levels.

Excito-Repellency Bioassay Procedure

Spatial repellency bioassays were conducted using a custom double-chamber excito-repellency apparatus featuring a cubic wooden main exposure chamber (23 × 23 × 23 cm) enclosed on four sides by a 52-mesh metal screen. The main chamber housed the treated substrate (fabric or paper) and connected to an untreated receiving cage via a sliding-door escape port. Mosquitoes were initially introduced into the exposure chamber with the port closed to allow for acclimation. Upon opening the port, specimens could either remain exposed to the treatment vapors within the main chamber or escape into the adjacent untreated receiving vessel.

1. Setup: The treated filter paper was lined along the inner walls of the main exposure chamber.
2. Introduction: Fifteen (15) active, non-blood-fed adult female Aedes aegypti were transferred into the main chamber using a mouth aspirator.
3. Acclimation: The escape port remained closed for a 3-minute adaptation period to acclimate the mosquitoes to the chamber conditions.
4. Observation: Following acclimation, the sliding door to the escape port was opened. The number of mosquitoes escaping from the treated main chamber into the receiving chamber was recorded at 1-minute intervals over a total period of 15 minutes.

Each treatment group was tested in triplicate, utilizing a fresh batch of mosquitoes and newly treated filter papers for every run to avoid behavioral bias or chemical residue carryover.

Data Gathering and Efficacy Calculations

At the conclusion of each 15-minute trial, the total number of escaped mosquitoes in the receiving chamber and the remaining mosquitoes in the main chamber were counted. Mosquitoes that successfully traversed the escape port were quantified to measure repellency and contact irritancy. The percentage escape rate (% Repellency) was computed for each trial using the following formula: Percent Repellency (%) = (N escaped / N total) x 100

Where N escaped represents the number of mosquitoes that exited into the receiving chamber, and N total is the total number of mosquitoes introduced per trial (N = 15).

Statistical Analysis

Experimental data were collected, tabulated, and processed using SPSS Statistics. Descriptive statistics, including means, were computed for escape rates across all seven treatment groups.

To determine whether statistically significant differences existed among the various extract concentrations, combination ratios, and controls, a One-Way Analysis of Variance (ANOVA) was performed. Assumptions of normality and homogeneity of variance were verified using the Shapiro-Wilk test and Levene's test, respectively. Where significant differences were identified ($p < 0.05$), Tukey's Honestly Significant Difference (HSD) post-hoc test was applied to conduct pairwise mean comparisons between treatment groups and establish relative efficacy against DEET.

Ethical Considerations and Biosafety Protocols

Because this study involved vector species capable of transmitting pathogen agents, biosafety procedures were strictly enforced. Non-human invertebrate subjects (A. aegypti) were handled according to institutional guidelines for laboratory biological safety.

Assay chambers were constructed from non-toxic materials, and test setups were double-contained inside mesh sleeve enclosures to prevent accidental vector escape into the environment. Following completion of the bioassays, all test mosquitoes, larvae, and biohazardous waste were euthanized via freezing at -20°C for 24 hours prior to autoclaving and final disposal in accordance with local environmental safety guidelines. The research team maintain transparency, avoided financial conflicts of interest, and cited all literature sources in accordance with APA guidelines.

RESULTS AND DISCUSSION

The evaluation of mosquito repellency across experimental treatments, benchmarked against positive and negative controls, demonstrated varying degrees of spatial escape responses in adult Aedes aegypti (Table 1). The negative control (untreated paper) yielded zero mosquito movement (0.00% repellency across all trials), confirming that movement within the chamber was driven entirely by chemical stimulation rather than mechanical handling stress. Conversely, the positive control (DEET) elicited the highest excito-repellent response, repelling a mean of 13.00 out of 15 mosquitoes per trial (86.67% mean repellency). This marked spatial avoidance aligns with established neurophysiological studies showing that DEET directly targets dedicated odorant receptors on A. aegypti antennal sensory neurons, disrupting olfactory signal coding and triggering immediate spatial avoidance (Stanczyk et al., 2013; Wang et al., 2026).

Table 1. Effectiveness of Psidium guajava and Basella rubra leaf extracts against adult Aedes aegypti (N = 15 per trial)

Treatment Group	Trial 1	Trial 2	Trial 3	Mean Repelled	Repellency in %
Positive Control (DEET)	13	13	13	13	86.67%
Negative Control (Untreated)	0	0	0	0	0
100% Psidium guajava	8	12	10	10	66.67%
100% Basella rubra	8	10	6	8	53.33%
50% P. guajava + 50% B. rubra	6	12	10	9.33	62.22%
25% P. guajava + 75% B. rubra	8	9	11	9.33	62.22%
75% P. guajava + 25% B. rubra	10	10	8	9.33	62.22%

Among the monotypic plant extracts, 100% Psidium guajava exhibited superior repellency compared to 100% Basella rubra, achieving a mean escape count of 10.00 mosquitoes (66.67% repellency) versus 8.00 mosquitoes (53.33% repellency), respectively. The enhanced spatial avoidance induced by P. guajava is primarily driven by its rich volatile profile, which contains high concentrations of monoterpenes and sesquiterpenes—that readily vaporize into the chamber air space and act as potent spatial irritants to mosquito olfactory apparatus (Edrolin, B. C., et al., 2024; Jhaiaun et al., 2021; Kartini & Sofia, 2022). In contrast, while B. rubra possesses a high concentration of non-volatile secondary metabolites like betacyanins, flavonoids, and saponins that exhibit contact toxicity and larvicidal activity (Chaurasiya et al., 2021; Dev Sudevan et al., 2024), its lower concentration of highly volatile lipophilic terpenes limits its ability to function effectively as a pure vapor-phase spatial repellent.

Interestingly, all three binary treatments —50% P. guajava + 50% B. rubra, 25% P. guajava + 75% B. rubra, and 75% P. guajava + 25% B. rubra— yielded an identical mean escape count of 9.33 mosquitoes (62.22% repellency). Blending B. rubra with P. guajava improved overall repellency relative to pure B. rubra (increasing escape rates from 53.33% to 62.22%), but none of the combination ratios surpassed the 66.67% repellency achieved by pure P. guajava. This pattern suggests an additive rather than a synergistic response, where incorporating B. rubra extract slightly diluted the vapor pressure of P. guajava's primary volatile odorants (Aremu et al., 2024; Wood et al., 2024). Nevertheless, the consistent 62.22% repellency maintained across all ratios indicates that even reduced proportions of P. guajava volatiles offer sufficient olfactory stimulation to induce moderate escape responses, providing a useful foundation for future formulation fixative studies.

Classifying the combined extracts' repellency (62.22%) as an additive interaction rather than a synergistic one reflects established pharmacodynamic criteria (Berenbaum, 1989; Chou, 2006). For true synergy to occur, a mixture must demonstrate bioactivity superior to its most potent individual constituent — a standard these blends did not achieve, as their repellency remained below that of unblended Psidium guajava (66.67%) (Tak

& Isman, 2015). Instead, the observed 62.22% escape rate closely mirrors the theoretical mathematical average of the two individual extracts (60.00%). This alignment demonstrates that Basella rubra's non-volatile metabolites failed to potentiate the vapor pressure or antennal receptor binding of guava's volatile monoterpenes and sesquiterpenes (Berenbaum, 1989). Rather, the volatile constituents of P. guajava exerted a straightforward, concentration-proportional influence on spatial escape behavior, which defines a linear additive response (Chou, 2006).

To evaluate whether variations in extract formulations and concentrations led to statistically meaningful differences in repellency, a One-Way Analysis of Variance (ANOVA) was performed across the seven experimental groups (Table 2). The statistical analysis revealed a highly significant difference in escape responses among the tested treatments ($p < 0.001$). Consequently, the null hypothesis stating that all formulation concentrations and controls exhibit equal repellent efficacy against adult Aedes aegypti was rejected.

Table 2. ANOVA Summary of the effectiveness of the Psidium guajava and Basella rubra leaf extracts against Aedes aegypti

Source of Variation	df	SS	MS	F stat	p-value	Decision
Between groups	6	302	50.33	16.18	9.1E-06	Reject Ho
Within groups	14	43.56	3.11			
Total	20	345.56				

This strong statistical significance is primarily driven by two key contrasts within the experimental dataset. First, the total absence of mosquito movement in the negative control (0.00% repellency) differed dramatically from all active treatment groups, confirming that the excito-repellent responses observed in the chambers were chemically induced rather than artifacts of mechanical handling (Wood et al., 2024). Second, the commercial benchmark DEET demonstrated significantly greater spatial repellency (86.67%) compared to both single and combined plant extracts. This gap in efficacy reflects fundamental differences in volatility kinetics: synthetic DEET maintains a high, constant vapor density that saturated the chamber's air space, whereas crude plant extracts release volatile compounds at lower, fluctuating vapor pressures (Lopez et al., 2025; Wang et al., 2026).

Furthermore, the significant variance between groups highlights the functional differences in chemical composition between Psidium guajava and Basella rubra. Monotypic P. guajava (66.67% repellency) outperformed monotypic B. rubra (53.33% repellency) due to its high concentration of volatile monoterpenes and sesquiterpenes, which readily volatilize and activate mosquito olfactory receptor neurons (Edrolin, B. C., et al., 2024; Jhaiaun et al., 2021; Kartini & Sofia, 2022). Conversely, while B. rubra extracts contain bioactive flavonoids and betacyanins that provide effective contact toxicity in larvicidal bioassays (Chaurasiya et al., 2021; Dev Sudevan et al., 2024; Krishnappa & Elumalai, 2013), their lower volatility results in reduced spatial repellency when deployed in a vapor-phase chamber setup. These ANOVA results confirm that while crude botanical extracts offer viable repellency, their efficacy varies significantly based on plant species selection and chemical volatility profiles (Aremu et al., 2024).

Applying a One-Way Analysis of Variance (ANOVA) to evaluate mosquito escape responses satisfied all core parametric assumptions. Independence of observations was maintained by introducing fresh, unexposed cohorts of 15 adult female Aedes aegypti per trial and thoroughly purging the test chamber with ethanol and acetone between replicates to prevent residual volatile accumulation (Stanczyk et al., 2013; Zar, 2010). The dependent variable— mean repellency expressed as a percentage of the total exposed population— provided continuous ratio-level data appropriate for parametric hypothesis testing (Field, 2013). Finally, strictly controlled ambient conditions (27°C, 75% relative humidity) limited extraneous within-group variance, while the balanced experimental structure ($n = 3$ replicates across all 7 treatment groups) ensured the F-statistic remained robust against minor departures from normality or variance homogeneity (Montgomery, 2019).

To evaluate the relative potency of each botanical formulation against the commercial benchmark, Tukey's Honestly Significant Difference (HSD) post-hoc pairwise comparison was conducted between each experimental treatment and the positive control (DEET) (Table 3).

Table 3. Recommended Concentration in repelling Aedes aegypti

Pairwise Comparison Treatment vs. DEET	Mean Difference (Treatment− DEET)	p-value	Statistical Significance (α=0.05)	Practical Recommendation
100% Psidium guajava	-3.67	0.1936	Not significant	Highly recommended
100% Basella rubra	-5.33	0.0249	Significant	Not recommended
50% P. guajava + 50% B. rubra	-4.33	0.0885	Not significant	Recommended
25% P. guajava + 75% B. rubra	-4.33	0.0885	Not significant	Recommended
75% P. guajava + 25% B. rubra	-4.33	0.0885	Not significant	Recommended

Among all plant-based treatments, monotypic 100% Psidium guajava demonstrated the closest performance to DEET, exhibiting a mean difference of -3.67 repelled mosquitoes ($p = 0.1936$). Because this difference was not statistically significant at 0.05 level, 100% P. guajava achieved spatial repellency statistically comparable to the synthetic standard. This strong performance is directly linked to the rich concentration of low-molecular-weight volatile monoterpenes and sesquiterpenes present in guava leaf extracts (Jhaiaun et al., 2021; Kartini & Sofia, 2022). These volatile compounds readily vaporize into the surrounding air, binding to and hyperstimulating olfactory receptor neurons on Aedes aegypti antennae to induce immediate excito-repellent avoidance behavior similar to DEET (Wang et al., 2026). Consequently, 100% P. guajava represents a highly recommended candidate for standalone botanical repellent formulations.

In contrast, monotypic 100% Basella rubra exhibited a statistically significant reduction in repellency compared to DEET, with a mean difference of -5.33 repelled mosquitoes ($p = 0.0249$). Because this p-value falls below the 0.05 significance threshold, the null hypothesis of equal efficacy was rejected for this group, confirming that pure B. rubra is significantly less effective than DEET as a vapor-phase repellent. Phytochemical profiles of B. rubra indicate that its bioactivity is concentrated primarily in non-volatile polyphenols, betacyanins, and flavonoids (Chaurasiya et al., 2021; Dev Sudevan et al., 2024). While these active metabolites exert potent larvicidal, ovicidal, and contact toxicity when ingested or applied topically (Aboshi et al., 2018; Reddy et al., 2023), their low vapor pressure prevents them from releasing sufficient gaseous odorants to drive spatial avoidance in adult mosquitoes. Therefore, unblended B. rubra is not recommended as a standalone spatial repellent.

Crucially, all three binary extract formulations— 50% P. guajava + 50% B. rubra, 25% P. guajava + 75% B. rubra, and 75% P. guajava + 25% B. rubra— demonstrated an identical mean difference of -4.33 repelled mosquitoes relative to DEET ($p = 0.0885$). Because these comparisons yielded p-values greater than 0.05, none of the combination blends differed significantly from DEET in spatial repellent efficacy. Incorporating even a minor fraction (25%) of P. guajava into B. rubra supplied sufficient volatile terpenoids to elevate overall spatial escape responses from 53.33% (pure B. rubra) to 62.22%, bridging the statistical performance gap relative to synthetic DEET (Aremu et al., 2024; Wood et al., 2024). These findings indicate that while pure guava provides the highest absolute repellency, binary formulations represent viable, recommended alternatives that can leverage B. rubra's non-volatile bioactive components for potential fixative or secondary protective properties (Lopez et al., 2025).

CONCLUSIONS

Bioassay results indicate that monotypic Psidium guajava (guava) leaf extract possesses strong spatial repellency against adult Aedes aegypti, while Basella rubra (Malabar spinach) extract exhibits minimal vapor-phase activity on its own. At 100% concentration, guava extract achieved 66.67% mean repellency, a performance statistically comparable to commercial DEET (86.67%). This spatial deterrence is driven primarily by volatile monoterpenes and sesquiterpenes within guava leaves, which readily vaporize into ambient air to trigger avoidance behavior in host-seeking mosquitoes. Conversely, pure B. rubra achieved only 53.33% repellency, performing significantly lower than DEET. Because the active secondary metabolites in B. rubra consist mainly of non-volatile flavonoids, betacyanins, and saponins, the crude extract lacks sufficient vapor pressure to induce strong spatial escape responses in a closed chamber setup.

Combining the two extracts across varying volumetric ratios (50:50, 25:75, and 75:25) produced a consistent repellency rate of 62.22%. Post-hoc statistical analysis confirmed that all three blend ratios performed on par with DEET, demonstrating that incorporating as little as 25% guava extract supplies enough volatile odorants to elevate overall spatial repellency. Although the combination ratios displayed an additive rather than synergistic effect— they successfully elevated B. rubra's repellent performance to a level statistically equivalent to the positive control. Consequently, guava leaf extract serves as an effective monotypic botanical candidate, and blending it with Malabar spinach offers a viable, stable alternative formulation.

RECOMMENDATIONS

Translating these preliminary laboratory findings into a viable botanical repellent requires addressing several formulation, chemical, and safety considerations. The immediate priority involves controlling the rapid evaporation rate inherent to plant-derived volatile terpenes. Future formulation development should evaluate the incorporation of natural chemical fixatives, such as vanillin or mineral oil bases, to sustain vapor release over extended periods. Testing these modified preparations in extended bioassays will help establish Complete Protection Time across realistic 4- to 8-hour windows. In parallel, comprehensive phytochemical profiling using Gas Chromatography-Mass Spectrometry (GC-MS) and High-Performance Liquid Chromatography (HPLC) should be conducted to isolate and quantify the specific monoterpenes and sesquiterpenes driving mosquito avoidance. Evaluating alternative extraction solvents along a polarity gradient—such as hexane or ethyl acetate—will further optimize the yield of active lipophilic compounds relative to non-volatile plant matrices.

Beyond chemical optimization, evaluating safety and real-world efficacy remains essential prior to consumer deployment. Before progressing to topical application, the extracts must undergo biomedical safety screening, including in vitro cytotoxicity testing on human dermal fibroblast cell lines and standardized patch assays to assess dermal irritation, allergic sensitization, skin absorption, and phototoxicity. Finally, evaluation must move past static laboratory chambers to standardized World Health Organization (WHO) arm-in-cage protocols and semi-field trials. Testing under variable environmental conditions—such as fluctuating temperatures, relative humidity, air currents, and human sweat factors—will determine how effectively the formulation performs against both laboratory-reared and wild-type field strains of Aedes aegypti.

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