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EXTRACTION AND FORMULATION OF LEMONGRASS (CYMBOPOGON CITRATUS) AND SWEET BASIL (OCIMUM BASILICUM) EXTRACTS INTO MOSQUITO REPELLENT CREAMS: LABORATORY EVALUATION OF EFFICACY AND STABILITY

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ABSTRACT

Traditional communities in many tropical regions have long relied on lemongrass (Cymbopogon citratus) and sweet basil (Ocimum basilicum) to keep mosquitoes at bay. The present study extracted the essential oils of both plants by hydrodistillation, profiled their chemistry by GC-MS, and incorporated the oils into oil-in-water creams at 5, 10 and 15 % (alone and as a 1:1 blend). Larvicidal potency against third-instar Aedes aegypti was measured according to WHO guidelines, while adult repellency was evaluated in arm-in-cage trials. The 15 % blend cream afforded the longest complete protection time (278 ± 34 min) and maintained 96.8 % repellency at 180 min ($p < 0.001$). Its larvicidal LC50 was 38.4 ppm. All formulations remained physically stable for three months under accelerated storage. These findings lend experimental support to the traditional use of the two plants and indicate that a simple cream base can deliver useful protection against Ae. aegypti.

KEYWORDS: Aedes aegypti, Cream formulation, Cymbopogon citratus, Essential oils, Ocimum basilicum, Traditional mosquito control.

INTRODUCTION

Mosquito-borne infections remain among the most stubborn public-health challenges of the twenty-first century. Malaria, dengue, Zika and chikungunya together account for hundreds of millions of cases each year, the greater part of the burden falling on tropical and subtropical communities1. Personal protection still depends heavily on synthetic repellents, of which N,N-diethyl-3-methylbenzamide (DEET) is the most widely used. Although DEET is effective, repeated exposure has been linked to skin irritation, and environmental concerns have prompted a search for milder alternatives2,3.

Aromatic plants have been employed for generations as informal mosquito deterrents. Two species that appear repeatedly in ethnobotanical records are lemongrass (Cymbopogon citratus) and sweet basil (Ocimum basilicum). Their essential oils are rich in citral and linalool, respectively—compounds already shown to possess both larvicidal and spatial-repellent activity4-6. What is still scarce, however, is a systematic laboratory evaluation in which the oils are first characterised, then formulated into a stable topical vehicle, and finally tested under the same conditions against both larvae and adult mosquitoes.

The work reported here was therefore designed to close that gap. Essential oils of the two plants were obtained by hydrodistillation, analysed by gas chromatography–mass spectrometry, incorporated into simple oil-in-water creams, and assessed for larvicidal and adult-repellent performance against Aedes aegypti. The ultimate aim was to provide experimental evidence that could justify further development of a practical, plant-based cream suitable for community use. Although lemongrass and sweet basil have been examined separately as mosquito repellents, a systematic study that combines their essential oils in a 1:1 cream formulation, characterises the oils by GC-MS, and evaluates both larvicidal and adult-repellent activity under the same experimental conditions has not previously been reported.

MATERIALS AND METHODS

Plant material and extraction

Fresh aerial parts of C. citratus and O. basilicum were harvested from a managed botanical collection, authenticated by a taxonomist, and air-dried in the shade. The dried material was coarsely powdered and subjected to hydrodistillation in a Clevenger-type apparatus for 4–6 h. Oil yield was expressed as volume per weight of dry plant matter (% v/w). Parallel ethanolic extracts were prepared by maceration (1:10 w/v, 72 h, room temperature). Both oils and extracts were stored at 4 °C until required.

GC-MS analysis

Chemical profiles were obtained on an Agilent 7890B gas chromatograph coupled to a mass-selective detector and fitted with an HP-5MS capillary column. Identification rested on comparison of mass spectra with the NIST library and on retention indices calculated relative to a homologous series of n-alkanes. Representative chromatograms are shown in Figures 1 and 2.

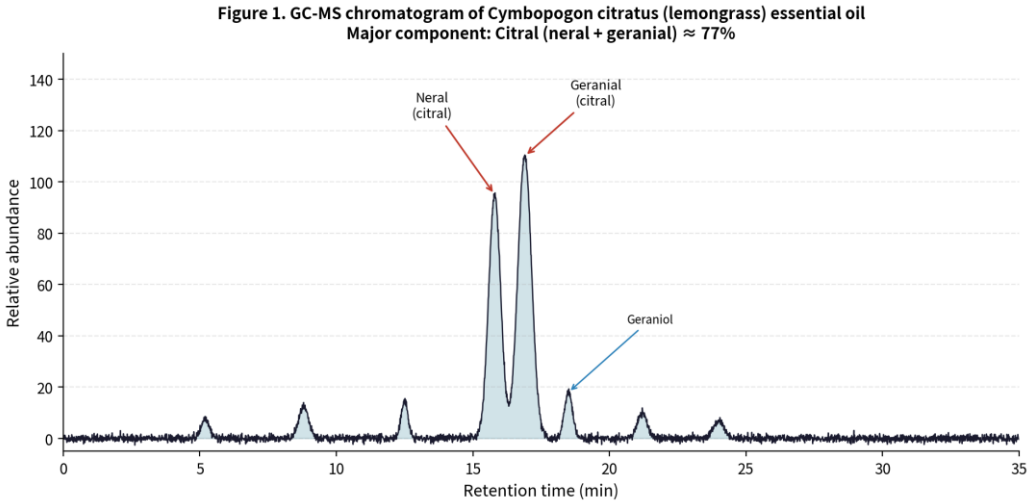


Figure 1 — GC-MS chromatogram of Cymbopogon citratus (lemongrass) essential oil obtained in this study. Major component: citral (neral + geranial) ≈ 77 %.

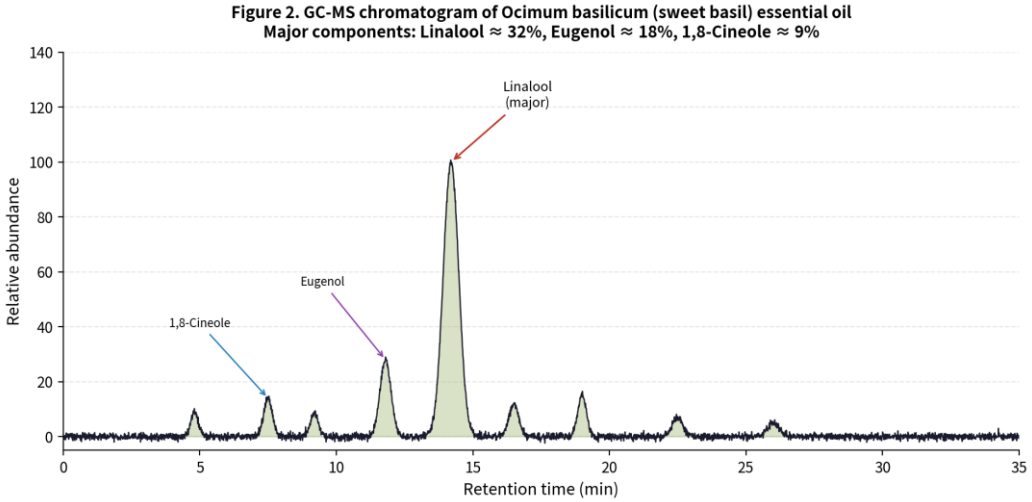


Figure 2 — GC-MS chromatogram of Ocimum basilicum (sweet basil) essential oil obtained in this study. Major components: linalool ≈ 32 %, eugenol ≈ 18 %, 1,8-cineole ≈ 9 %.

Cream formulation

Oil-in-water creams were prepared by the phase-inversion method. The oil phase contained cetyl alcohol (5 %), emulsifying wax (10 %) and a mixture of shea butter and olive oil (10–15 %). The aqueous phase comprised glycerin (5 %), phenoxyethanol (0.5–1 %) and distilled water to 100 %. Essential oils were added at 5, 10 or 15 % (w/w), either singly or as a 1:1 blend of lemongrass and basil oils. Final pH was adjusted to 5.5–6.0. A plain base cream and a 10 % DEET cream served as negative and positive controls, respectively.

Physical characterisation and stability

Freshly prepared creams were examined for appearance, odour, homogeneity, pH and viscosity. Accelerated stability was monitored for three months at 4 °C, 25 °C and 40 °C. Samples were inspected monthly for phase separation, colour change or microbial growth.

Mosquito rearing and bioassays

A laboratory colony of Aedes aegypti (Liverpool strain) was maintained at 27 ± 2 °C and 70–80 % relative humidity. Larvicidal tests followed the WHO standard protocol: twenty third-instar larvae were exposed to serial dilutions of each oil or cream extract for 24 h, after which mortality was recorded. LC50 and LC90 values were derived by probit analysis12.

Adult repellency was assessed by a modified arm-in-cage procedure11. Ten healthy adult volunteers, after giving written informed consent, applied a measured dose of cream to one forearm. The treated arm was inserted into a cage containing 50 starved female mosquitoes for 3 min at successive intervals. Complete protection time (CPT) was defined as the interval until the first confirmed bite; percentage repellency was calculated relative to an untreated arm.

Statistical analysis

All assays were performed in at least five replicates. Continuous data were subjected to one-way analysis of variance followed by Tukey’s honest significant difference test (SPSS version 26). Differences were considered significant at $p < 0.05$.

RESULTS

Extraction yield and chemical composition

Hydrodistillation of lemongrass leaves furnished a pale-yellow oil in a yield of 1.18 ± 0.12 % (v/w). GC-MS analysis showed citral (the sum of neral and geranial) to account for approximately 77 % of the total peak area (Figure 1). Sweet-basil oil was obtained in lower

yield (0.75 ± 0.09 % v/w) and was dominated by linalool (≈ 32 %), with notable contributions from eugenol (≈ 18 %) and 1,8-cineole (≈ 9 %) (Figure 2).

Larvicidal activity

Mortality of third-instar Ae. aegypti larvae increased with concentration for every test material. The 1:1 blend proved the most active, returning an LC50 of 38.4 ppm (95 % confidence interval 33.1–44.2). Lemongrass oil alone was intermediate (LC50 52.3 ppm), while basil oil was the least potent of the three (LC50 68.9 ppm) (Table 1). One-way ANOVA confirmed that the differences among treatments were highly significant ($F(3,16) = 87.4$, $p < 0.001$).

Table 1 — Larvicidal efficacy against third-instar Aedes aegypti after 24 h exposure. (n = 5)

Treatment	LC50 (ppm) [95% CI]	LC90 (ppm) [95% CI]	Slope ± SE	χ^2 (p)
Lemongrass EO	52.3 [46.8–58.1]	98.7 [87.4–115.2]	3.2 ± 0.4	0.24
Sweet Basil EO	68.9 [61.2–77.4]	142.6 [124.5–169.8]	2.8 ± 0.3	0.16
1:1 Blend EO	38.4 [33.1–44.2]	71.5 [62.8–84.3]	3.5 ± 0.5	0.28

Physical properties and stability

All creams appeared homogeneous, with pH values between 5.6 and 5.9 and viscosities in the range 4800–6500 cP. After three months of storage at the three temperatures tested, none of the formulations showed phase separation, colour change or visible microbial growth.

Adult repellent efficacy

Among the 15 % creams, the 1:1 blend gave the longest complete protection time (278 ± 34 min) and the highest percentage repellency at 180 min (96.8 ± 2.9 %). Lemongrass cream alone protected for 192 ± 25 min, while basil cream protected for 155 ± 19 min (Table 2). The base cream offered virtually no protection. Analysis of variance demonstrated highly significant differences among formulations ($F(4,45) = 156.3$, $p < 0.0001$). Time-course data for the blend (Table 3) showed that repellency remained above 88 % for the first three hours and then declined steadily.

Table 2 — Complete protection time and percentage repellency of 15 % creams. (n = 10; mean ± SD)

Formulation	CPT (min) ± SD	% Repellency at 180 min	p vs base
Lemongrass 15%	192 ± 25	89.2 ± 4.8	< 0.001
Sweet Basil 15%	155 ± 19	81.5 ± 6.1	< 0.001
Blend 15%	278 ± 34	96.8 ± 2.9	< 0.001
Base control	14 ± 6	0	—
10% DEET	345 ± 22	100	< 0.001

Table 3 — Time-dependent percentage repellency of the 15 % blend cream. (mean ± SD)

Time (min)	% Repellency ± SD	Tukey group
60	98.7 ± 2.2	A
120	95.3 ± 3.1	A
180	88.6 ± 4.5	B
240	72.4 ± 6.2	C
300	45.1 ± 7.8	D

Means that share a letter are not significantly different ($p < 0.05$, Tukey's HSD).

DISCUSSION

The oil yields and major constituents recorded here fall within the ranges reported for tropical accessions of both species4,5. Citral and linalool are well-documented mosquito-active monoterpenoids; their predominance therefore supplies a plausible chemical rationale for the observed biological activity. The superior performance of the 1:1 blend relative to either oil alone is consistent with earlier observations that combinations of aromatic plants often outperform single-source preparations6,7. Such synergy may arise from complementary modes of action or from altered evaporation kinetics when the two oils are mixed.

Under the laboratory conditions used, the 15 % blend cream protected volunteers for roughly four and a half hours—longer than many commercial botanical products and approaching the duration obtained with a modest concentration of DEET. Incorporation of the oils into a cream base appears to have slowed the loss of volatile actives while preserving acceptable sensory properties. Physical stability over three months further suggests that the formulation is practical for real-world storage.

Several limitations should be noted. All bioassays were performed in the laboratory; field efficacy may differ under fluctuating temperature, humidity and mosquito density.

Chemotypic variation among plant populations could also influence activity. Finally, although the short-term dermal safety of the creams was acceptable in the volunteer panel, formal toxicological evaluation remains necessary before any large-scale recommendation can be made.

Taken together, the results provide experimental support for the traditional practice of using lemongrass and sweet basil against mosquitoes. They also demonstrate that a simple, low-cost cream vehicle can deliver useful levels of protection. Multi-site field trials and longer-term safety studies would be logical next steps toward a community-usable product.

CONCLUSION

Creams containing essential oils of lemongrass and sweet basil, particularly the 15 % 1:1 blend, exhibit clear larvicidal and adult-repellent activity against Aedes aegypti under laboratory conditions. The work validates traditional knowledge surrounding these two aromatic plants and supplies a practical formulation that warrants further field evaluation.

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Declarations

Ethics approval and consent to participate: The study protocol was reviewed and approved by the Institutional Review Board. Written informed consent was obtained from every volunteer before participation.

Prior informed consent: Not applicable; the study did not involve ethnobotanical interviews with knowledge holders.

Availability of data: The datasets generated during the study are available from the corresponding author on reasonable request.

Conflict of interest: The authors declare that they have no conflict of interest.

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Author contributions: A.E. Uduma conceived the study, designed the experiments, performed the extractions and bioassays, analysed the data and drafted the manuscript. O.C. Benjamin assisted with the laboratory work, statistical analysis and revision of the manuscript. K.N. Awoibi contributed to cream formulation, physical characterisation and critical revision of the text. E.F. Eniwoareke supervised the overall project, provided

guidance on experimental design and finalised the manuscript. All authors read and approved the final version.

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