

Malaria-repellent medicinal plants in Ethiopia: Indigenous knowledge and bioactive potential

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Abstract

Malaria remains a major public health burden in Ethiopia, particularly in rural regions where access to synthetic repellents is limited. Indigenous communities have traditionally used medicinal plants for mosquito protection, yet scientific validation of these practices remains insufficient. This study investigated the chemical composition, repellent efficacy, and practical applicability of four Ethiopian medicinal plants, *Cymbopogon citratus*, *Ocimum suave*, *Azadirachta indica*, and *Eucalyptus globulus*, against *Anopheles* mosquitoes, comparing their performance to DEET. Findings: Phytochemical analysis revealed citronellal as the most abundant compound (65% total accumulation). Laboratory tests showed *C. citratus* and *O. suave* achieved >90% initial repellency, comparable to DEET (97.2%). Field trials in Dire Dawa, Harari, and Somali regions demonstrated 75% and 70% bite reduction over two hours, respectively. Statistical analysis confirmed significant differences among treatments ($F(4,95) = 217.48, p < 0.001$) with a large effect size ($\eta^2 = 0.9015$). A strong positive correlation was observed between citronellal concentration and efficacy ($r = 0.834$). *Cymbopogon citratus* and *Ocimum suave* are effective, accessible alternatives to synthetic repellents for malaria prevention in rural Ethiopia. These plants should be integrated into community-based malaria control programs, with standardized formulations and further commercialization studies.

Keywords: malaria, medicinal plants, indigenous knowledge, bioactive, Ethiopia

Plantas medicinais repelentes de malária na Etiópia: conhecimento indígena e potencial bioativo

Resumo

A malária continua a representar um importante problema de saúde pública na Etiópia, particularmente nas regiões rurais, onde o acesso a repelentes sintéticos é limitado. As comunidades indígenas utilizam tradicionalmente plantas medicinais para proteção contra mosquitos; contudo, a validação científica dessas práticas ainda é insuficiente. Este estudo investigou a composição química, a eficácia repelente e a aplicabilidade prática de quatro plantas medicinais etíopes, *Cymbopogon citratus*, *Ocimum suave*, *Azadirachta indica* e *Eucalyptus globulus* contra mosquitos do gênero *Anopheles*, comparando seu desempenho com o repelente sintético DEET. A análise fitoquímica revelou que o citronelal foi o composto mais abundante, representando 65% da acumulação total. Os ensaios laboratoriais demonstraram que *C. citratus* e *O. suave* alcançaram repelência inicial superior a 90%, comparável ao DEET (97,2%). Nos ensaios de campo realizados nas regiões de Dire Dawa, Harari e Somali, essas espécies reduziram as picadas de mosquitos em 75% e 70%, respectivamente, durante um período de duas horas. A análise estatística confirmou diferenças significativas entre os tratamentos ($F(4,95) = 217,48; p < 0,001$), com um grande tamanho de efeito ($\eta^2 = 0,9015$). Observou-se ainda uma forte correlação positiva entre a concentração de citronelal e a eficácia repelente ($r = 0,834$). *Cymbopogon citratus* e *Ocimum suave* constituem alternativas

eficazes, acessíveis e sustentáveis aos repelentes sintéticos para a prevenção da malária nas zonas rurais da Etiópia. Recomenda-se a integração dessas plantas em programas comunitários de controlo da malária, acompanhada do desenvolvimento de formulações padronizadas e de estudos adicionais visando à sua comercialização.

Palavras-chave: malária, plantas medicinais, conhecimento indígena, compostos bioativos, Etiópia

1. Introduction

Malaria remains a major global health concern, affecting over 241 million people annually and leading to approximately 627,000 deaths worldwide, with sub-Saharan Africa bearing a significant portion of this burden (WHO, 2021). In Ethiopia, malaria is one of the most prevalent public health issues, particularly in rural areas, where access to healthcare and preventive resources is limited. The country experiences high rates of malaria transmission in lowland areas, with over 60% of the population at risk of contracting the disease (Ministry of Health, Ethiopia, 2022). This situation places a strain on both health systems and the socio-economic stability of affected communities, as malaria often leads to reduced productivity, increased healthcare costs, and higher rates of absenteeism in the workforce and schools (Deressa et al., 2020). Given these impacts, malaria prevention remains an urgent priority, especially in endemic regions where traditional healthcare infrastructure may fall short.

While several preventive strategies have been employed, including insecticide-treated bed nets and indoor residual spraying, these measures alone are insufficient to fully prevent mosquito bites and subsequent malaria transmission (Tadele et al., 2019; Goshu, 2026). The use of repellents, which provide a protective barrier against mosquitoes, is widely recognized as an essential component of malaria prevention, as repellents can directly reduce the frequency of mosquito-human contact (Charavanamuttu et al., 2026; Govella; Ferguson, 2019).

However, access to synthetic repellents remains limited in many parts of Ethiopia, especially in rural communities where high costs and environmental concerns may hinder their regular use. Moreover, there is growing awareness of the potential adverse health effects and environmental consequences associated with the continuous use of chemical-based repellents (Deressa et al., 2020). Consequently, there is a pressing need to explore alternative, locally sourced, and eco-friendly options to complement existing malaria prevention strategies.

Indigenous medicinal plants hold significant promise in this regard, as they have been used by local communities for centuries as natural repellents against mosquitoes. Ethiopian ethnobotanical studies indicate that rural populations often rely on medicinal plants to protect themselves from mosquito bites, utilizing a variety of plant species with known mosquito-repellent properties (Abebe; Gemedo-Dalle, 2021). However, despite the widespread traditional use of these plants, scientific research on their efficacy and the identification of active compounds remains limited. This gap in current research underscores the importance of investigating the effectiveness of these indigenous plants, both to validate traditional knowledge and to potentially develop sustainable, plant-based repellent products that can contribute to malaria prevention efforts in Ethiopia and beyond (Abate et al., 2021).

In light of the high burden of malaria in Ethiopia and the limitations of existing preventive methods, this study aims to explore and document the repellent potential of selected Ethiopian medicinal plants. By identifying and analyzing their bioactive compounds, this research seeks to bridge the gap between indigenous knowledge and modern malaria control strategies, with the goal of providing accessible, eco-friendly alternatives for communities vulnerable to malaria transmission.

1.1 Statement of the problem

Malaria remains one of the most pressing public health challenges in Ethiopia, with an especially high prevalence in the Somali region, East and West Hararghe, and Harari regions. These areas, characterized by a predominantly rural population, face limited access to healthcare services, leading to increased vulnerability to malaria transmission. In these regions, malaria not only contributes to morbidity and mortality but also exacerbates economic hardships by reducing workforce productivity and increasing healthcare costs (Deressa et al., 2020). The burden of malaria is particularly severe in rural settings, where limited infrastructure, poverty, and geographical isolation hinder access to preventative resources, including synthetic mosquito repellents. Consequently, there is an urgent need for effective, affordable, and locally accessible malaria prevention solutions in these communities.

Although synthetic repellents are known to be effective, they remain largely unavailable or unaffordable for rural populations in Ethiopia. High costs, logistical challenges in distribution, and concerns over potential health and environmental impacts associated with chemical-based repellents limit their use among economically disadvantaged communities (Tadele et al., 2019). Furthermore, these synthetic repellents often come with limited shelf life and require consistent reapplication, adding to the logistical and financial burden on users. In regions such as Somali, Hararghe, and Harari, where traditional practices and reliance on natural resources are ingrained in daily life, there is a pressing need for alternative repellents that are both culturally acceptable and readily available (Alemayehu; Getachew, 2021; Goshu, 2026).

Indigenous medicinal plants have long been used by local communities in Ethiopia as natural repellents, yet these practices are frequently based on traditional knowledge without formal scientific validation or standardization. Ethnobotanical studies indicate that numerous plants endemic to Ethiopia possess mosquito-repellent properties, which are leveraged by communities in rural areas to combat malaria (Abebe; Gemedo-Dalle, 2021). However, despite this promising traditional knowledge, there is a significant research gap regarding the efficacy, safety, and optimal application of these plants as mosquito repellents. This lack of empirical evidence prevents broader adoption of plant-based repellents and limits their potential to serve as a sustainable and accessible solution for malaria prevention in regions where synthetic repellents are inaccessible or unaffordable (Alemayehu et al., 2021).

The current reliance on chemical repellents, coupled with the need for sustainable, eco-friendly alternatives, underscores the importance of scientifically exploring and validating the repellent properties of Ethiopian medicinal plants. By doing so, this research seeks to address a critical gap in malaria prevention efforts in rural Ethiopia, offering communities in Somali, East, and West Hararghe, and Harari regions a practical, cost-effective, and environmentally friendly means of protection against malaria. In the absence of accessible synthetic options, these plant-based repellents could provide a vital resource for malaria control, aligning with both community practices and ecological sustainability goals.

General objective

The main purpose of this study is to evaluate the efficacy of selected medicinal plants from Dire Dawa, East Harerge, West Harerge (Oromiya), Harari, and Somali regions in Ethiopia in repelling malaria vectors and to identify their active compounds. The specific objectives are

- To document the traditional use of specific medicinal plants for malaria prevention in Dire Dawa, East Harerge, and West Harerge (Oromiya), Harari, and Somali regions.
- To extract and analyze the active compounds in these plants from the specified regions.
- To assess the effectiveness of these compounds in repelling malaria vectors in comparison to commercial repellents.

Research questions

1. Which medicinal plants are commonly used for malaria prevention in Dire Dawa, East Harerge, West Harerge (Oromiya), Harari, and Somali regions?
2. What are the bioactive compounds responsible for the repellent properties of these plants?
3. How effective are these plants in comparison to commercial repellents in the specified regions?

2. Literature Review

2.1 Global teacher shortage and its impact on education in malaria-prone regions

Malaria remains a major global health challenge, with an estimated 247 million clinical cases and approximately 619,000 deaths in 2021, with sub-Saharan Africa bearing a disproportionate burden of this morbidity and mortality (WHO, 2021; Shibeshi et al., 2024). In Ethiopia, malaria is endemic in lowland areas, placing over 60% of the population at risk, particularly in rural regions such as Dire Dawa, East and West Hararge, Harari, and Somali areas, where healthcare infrastructure and preventive resources are limited (Ministry of Health, Ethiopia, 2022). The socioeconomic impact of malaria in these regions is profound, contributing to reduced productivity, increased healthcare expenditure, and heightened rates of school and workforce absenteeism (Deressa et al., 2020).

The WHO recommends two primary vector control interventions for large-scale malaria prevention: insecticide-treated nets (ITNs) and indoor residual spraying (IRS) (WHO, 2023, 2024). However, these measures

alone are insufficient to fully interrupt malaria transmission, particularly in areas where *Anopheles* mosquitoes exhibit outdoor biting behaviors or resistance to pyrethroid insecticides (Tadele et al., 2019; Govella; Ferguson, 2019). Recent WHO guidelines (2023, 2024) emphasize the need for integrated approaches that combine multiple interventions, including personal protective measures such as repellents, to achieve maximum impact in resource-constrained settings. The emergence of insecticide resistance in malaria vectors across Ethiopia and other endemic regions has further stressed the urgent need for developing and implementing alternative, sustainable vector control measures (Goshu, 2026; Alemayehu; Getachew, 2021; Shibeshi et al., 2024).

2.2 Integrated vector management and the role of botanical repellents

The concept of Integrated Vector Management (IVM) was developed by the WHO as a rational decision-making process that encourages optimal use of resources for efficient, cost-effective, and sustainable vector control (WHO, 2023). IVM emphasizes the integration of multiple interventions, including environmental management, biological control, chemical methods, and personal protection, tailored to local epidemiological and entomological contexts. Within this framework, personal repellents play a critical complementary role, particularly in reducing human-vector contact during peak biting hours when ITNs and IRS may be less effective (Govella; Ferguson, 2019).

Botanical repellents derived from medicinal plants have gained increasing attention as components of IVM strategies, offering biodegradable, environmentally friendly, and locally accessible alternatives to synthetic chemicals (Kaushik et al., 2023). A systematic review by Kaushik et al. (2023) demonstrated that essential oils and extracts from diverse plant families, including *Azadirachta*, *Calotropis*, *Cinnamomum*, *Citrus*, *Eucalyptus*, *Geranium*, *Mentha*, *Lantana*, *Ocimum*, *Piper*, and *Zingiber*, exhibit significant mosquito-repellent activities, with modes of action involving olfactory receptor interference and behavioral disruption. Recent research has further highlighted the potential of nanoemulsion-based formulations to enhance the controlled release and stability of plant-derived repellents, offering promising avenues for improved malaria control (AnnaDurai et al., 2024; Kantheti; Gupta, 2023).

2.3 Traditional use of medicinal plants for malaria prevention in Africa

In Africa, the use of medicinal plants for mosquito protection is deeply embedded in cultural and traditional practices, with knowledge transmitted across generations (Mujovo et al., 2020). Across Ethiopia, rural communities have long relied on locally available plants to repel mosquitoes and reduce malaria risk (Abebe, 2019). Ethnobotanical studies in the Somali Regional State of eastern Ethiopia documented 42 plant species used as repellents against mosquitoes, with *Amaranthus sparganiocephalus*, *Phytolacca dodecandra*, and *Commiphora rostrata* among the most frequently cited (Alemayehu et al., 2024; Hammer; van der Maesen, 2025; Misganaw; Asfaw, 2014).

In the Seweyna district of southeastern Ethiopia, a recent ethnobotanical study documented 19 plant species from 12 families used as mosquito repellents, with *Mimusops kummel* (90.81%), *Acokanthera schimperi* (84.69%), *Boswellia microphylla* (79.6%), and *Calpurnia aurea* (79.6%) being the most frequently mentioned (Shibeshi et al., 2024). The stem was the most common plant part used (47.3%), and most communities (52.6%) employ burning of fresh or dry plant parts to generate smoke as the primary practice (Shibeshi et al., 2024).

Beyond Ethiopia, research across sub-Saharan Africa has consistently demonstrated the repellent potential of indigenous plants. In Burkina Faso, five essential oils extracted from local plants, including *C. citratus*, *Cymbopogon nardus*, and *Eucalyptus camaldulensis*, produced repellent-irritant effects against *Anopheles gambiae* comparable to DEET (Bationo et al., 2024). Field evaluations in the Bolivian Amazon confirmed that *C. citratus* essential oil at 25% provided 74% protection for 2.5 hours against *Anopheles darlingi*, demonstrating the practical applicability of plant-based repellents in real-world settings (Moore et al., 2007). Importantly, plant-based repellents, though often less potent than synthetic alternatives, were shown to be more culturally acceptable and affordable, potentially enhancing user compliance among marginalized populations (Moore et al., 2007).

2.4 Bioactive compounds in malaria-repellent plants

The efficacy of medicinal plants as mosquito repellents is primarily attributed to bioactive secondary metabolites, particularly terpenoids, phenylpropanoids, and limonoids, which interfere with insect olfactory receptors or exert

toxic effects (Nerio et al., 2010; Kaushik et al., 2023). Among the most extensively studied compounds are citronellal, eugenol, limonene, 1,8-cineole, and azadirachtin.

Citronellal, a monoterpenoid aldehyde abundant in *Cymbopogon* species (lemongrass), is widely recognized for its potent repellent activity against *Anopheles* mosquitoes (Kebede et al., 2020). Recent studies have confirmed that the repellent-irritant effects of *C. citratus* essential oil against *Anopheles gambiae* are very close to those of DEET (Bationo et al., 2024). Field evaluations have demonstrated that *C. citratus* essential oil at 25% provides 74% protection for 2.5 hours against *Anopheles darlingi* (Moore et al., 2007). Similarly, eugenol, a phenylpropanoid found in *Ocimum* species, exhibits strong repellent and insecticidal properties, while 1,8-cineole (eucalyptol), prevalent in *Eucalyptus* species, has shown effectiveness in both repelling and incapacitating malaria mosquitoes (Dube et al., 2019; Fekadu; Mesfin, 2022; Kantheti; Gupta, 2023).

Azadirachtin, a limonoid unique to *Azadirachta indica* (neem), is renowned for its antifeedant, growth-disrupting, and repellent properties against a wide range of insects, including *Anopheles* mosquitoes (Okumu; Moore, 2017; 2023). Neem-based products have demonstrated ovicidal, larvicidal, pupicidal, and repellent effects against mosquitoes of medical importance, including *Anopheles* species (Benelli et al., 2017; Chandramohan et al., 2016). The synergistic presence of multiple terpenoids in many plant species suggests multi-target mechanisms of action, potentially enhancing overall repellent efficacy and reducing the likelihood of resistance development (Nerio et al., 2010; Kaushik et al., 2023).

Recent research in Ethiopia has further expanded the inventory of bioactive plant species. The essential oil of *Leucas stachydiformis*, an endemic Ethiopian plant, demonstrated larvicidal, ovicidal, and repellent activities against *Anopheles arabiensis*, the dominant malaria vector in the country, with a total percentage protection of 67.9% at 366.7 µg/cm² over four hours (Fikru et al., 2024). These findings underscore the rich potential of Ethiopian flora for developing plant-based malaria vector control strategies.

2.5 Limitations of synthetic repellents and the case for botanical alternatives

While synthetic repellents, particularly DEET (N, N-diethyl-meta-toluamide), are highly effective in reducing mosquito-vector contact, their widespread use in rural Ethiopia is constrained by economic, health, and environmental concerns. DEET, though the gold-standard repellent, can cause skin irritation and allergic reactions in sensitive individuals, and there is growing concern about its potential neurotoxicity with prolonged exposure (Curtis; Lines, 2000). Moreover, synthetic repellents are often prohibitively expensive for rural households, leading to inconsistent use and reduced protective coverage (Kebede et al., 2020).

Environmental persistence is another significant concern. Chemical residues from synthetic repellents may contaminate water sources and pose risks to non-target organisms, including beneficial insects and aquatic life (Tamaru et al., 2018). Furthermore, the continuous use of synthetic chemicals has contributed to the emergence of insecticide resistance in mosquito populations, undermining the long-term effectiveness of vector control programs (Alemayehu; Getachew, 2021; Goshu, 2026; Bationo et al., 2024).

In contrast, plant-based repellents offer several advantages: they are biodegradable, environmentally friendly, and can be locally cultivated and processed, reducing dependence on imported chemicals (Kaushik et al., 2023; AnnaDurai et al., 2024). Their cultural acceptability and alignment with traditional practices further enhance community uptake and sustained use (Mujovo et al., 2020; Moore et al., 2007; Shibeshi et al., 2024). The WHO has increasingly recognized the role of locally sourced, eco-friendly interventions in achieving sustainable malaria control, particularly in resource-limited settings (WHO, 2021, 2023). Given these considerations, promoting the use of indigenous medicinal plants as alternatives or supplements to synthetic repellents aligns with both public health goals and environmental sustainability objectives.

2.6. Research Gaps and Rationale for the Present Study

Despite the widespread traditional use of medicinal plants for mosquito protection in Ethiopia, scientific validation of their efficacy, safety, and optimal application remains limited. While ethnobotanical surveys have documented numerous plant species with perceived repellent properties (Shibeshi et al., 2024; 2023), systematic comparative studies evaluating their chemical composition, repellent efficacy under controlled and field conditions, and performance relative to synthetic standards are scarce (Abebe; Gemedo-Dalle, 2021; Abate et al., 2021). Furthermore, the identification of specific bioactive compounds responsible for repellent activity and the elucidation of their mechanisms of action require further investigation.

The present study addresses these gaps by comprehensively evaluating the chemical composition, repellent efficacy, and practical applicability of four Ethiopian medicinal plants: *Cymbopogon citratus*, *Ocimum suave*, *Azadirachta indica*, and *Eucalyptus globulus*, against *Anopheles* mosquitoes. By integrating phytochemical analysis, laboratory bioassays, and field trials in malaria-endemic regions of eastern Ethiopia, this research seeks to bridge the gap between indigenous knowledge and evidence-based malaria control, providing a foundation for the development of accessible, sustainable, and culturally appropriate repellent interventions.

3. Materials and Methods

This study aimed to evaluate the efficacy of selected Ethiopian medicinal plants from Dire Dawa, Oromia region (East and West Hararge), Harari region, and the Somali region as mosquito repellents against malaria vectors. The following sections provide a detailed description of the study design, including sample selection, plant collection, extraction methods, repellent testing, and data analysis methods.

3.1 Study area

The research was conducted in Dire Dawa, East and West Hararge in the Oromia region, Harari region, and the Somali region of Ethiopia. These areas are highly vulnerable to malaria, particularly in rural zones where access to synthetic repellents is limited due to high costs or availability issues (WHO, 2021). Dire Dawa and Harari have semi-arid climates, with temperatures conducive to mosquito breeding, particularly during the rainy season. East and West Hararge and parts of the Somali region are also prone to malaria outbreaks due to seasonal rainfall and the presence of stagnant water bodies that create breeding grounds for mosquitoes. These regions are also culturally diverse, with unique traditional knowledge of medicinal plants that are thought to have repellent properties. This diversity supports ethnobotanical exploration and provides a basis for examining various plants used in traditional malaria prevention practices (Abebe, 2019).

Figure 1 illustrates the geographical distribution of the study areas across Dire Dawa, East and West Hararge (Oromia Region), Harari Region, and the Somali Region of eastern Ethiopia. These locations were selected because of their high malaria vulnerability, favorable ecological conditions for *Anopheles* mosquito breeding, and rich ethnobotanical diversity. The semi-arid climate, seasonal rainfall, and widespread stagnant water bodies create ideal conditions for malaria transmission (WHO, 2021). Moreover, the cultural diversity of these regions provides extensive indigenous knowledge regarding medicinal plants traditionally used as mosquito repellents, supporting ethnopharmacological investigation and the identification of locally available bioactive species (Abebe, 2019).

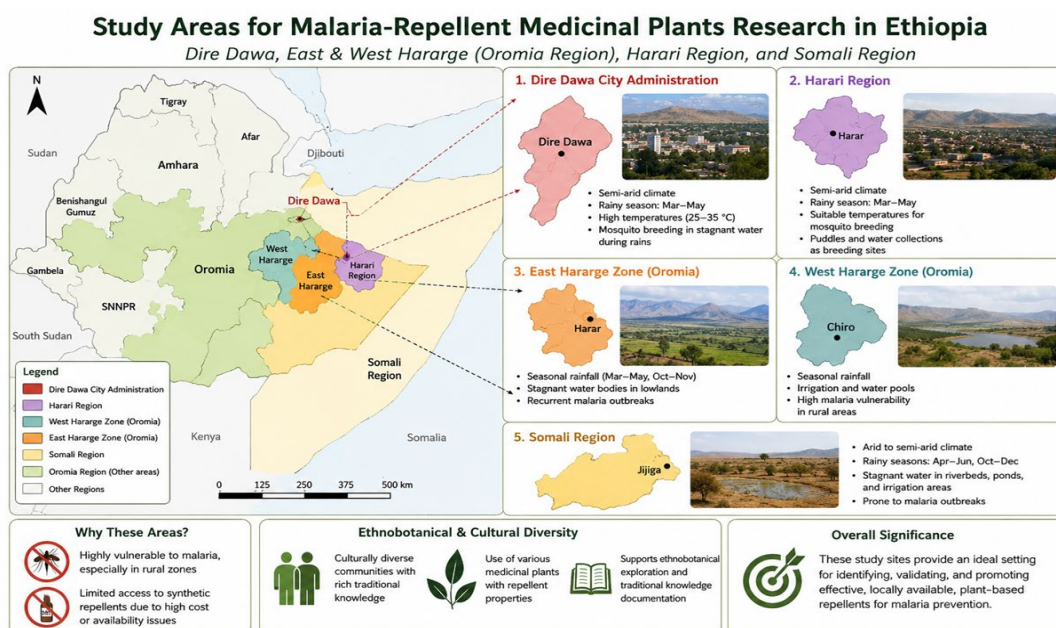


Figure 1. Geographic distribution of the Ethiopian study areas highlighting malaria-prone environments, ecological suitability, and ethnobotanical diversity for medicinal-plant research. Source: Authors, 2026.

3.2 Sample selection and plant collection

A purposive sampling approach was employed to select plant species based on their traditional use for malaria prevention. Initial data on these plants were gathered through ethnobotanical surveys conducted in consultation with local healers, traditional medicine practitioners, and community elders, who possess indigenous knowledge about the plants commonly used as repellents in these regions. This information was documented through semi-structured interviews and focus group discussions to obtain a comprehensive understanding of plant use, preparation, and application methods (Kebede et al., 2020). Key selection criteria included the frequency of plant use, availability, and anecdotal evidence of efficacy against mosquitoes. At least five to ten plant species were targeted for collection to allow for comparative analysis; ultimately, four species (*C. citratus*, *O. suave*, *A. indica*, and *E. globulus*) were selected based on their prominence in the ethnobotanical surveys.

Following the ethnobotanical survey, plants were collected from the specified regions under ethical guidelines, ensuring minimal impact on local biodiversity. The collection was conducted at the peak growth season to maximize the concentration of active compounds. Plant parts with known repellent properties, such as leaves, stems, or roots, were gathered, documented, and properly labeled for later identification. Collected samples were transported to the laboratory under controlled conditions to preserve their integrity (Fekadu; Mesfin, 2022).

3.3 Preparation, extraction, and characterization of essential oils

The preparation and extraction of bioactive compounds from the collected plant samples were conducted in two primary phases: drying and extraction.

Drying: Plant materials were washed to remove contaminants and then air-dried in a shaded, well-ventilated area for seven to ten days. Air drying under shade was preferred to avoid direct sunlight, which can degrade heat-sensitive bioactive compounds (Dube et al., 2019). After complete drying, the plant materials were ground into a fine powder using an electric grinder.

Extraction: The extraction process utilized both steam distillation and solvent extraction methods to isolate the volatile and non-volatile compounds responsible for repellent properties.

Steam Distillation: This method was applied to plants with high levels of volatile compounds (*C. citratus*, *O. suave*, and *E. globulus*). Dried plant material (200 g per batch) was placed in a Clevenger-type distillation apparatus, and steam passed through it for 3–4 h to separate the essential oils. The resulting distillate was collected, dried over anhydrous sodium sulfate, and stored in amber vials at 4 °C until testing (Alemayehu et al., 2021). The extraction yields (% w/w) were calculated as (mass of essential oil/mass of dried plant material) × 100. The yields were as follows: *C. citratus*: 0.82% ± 0.05%; *O. suave*: 0.71% ± 0.04%; and *E. globulus*: 1.15% ± 0.06%.

Solvent Extraction: For *A. indica*, which contains less volatile limonoid compounds, solvent extraction was employed. Ground leaves (100 g) were macerated in 500 mL of 95% ethanol for 48 h at room temperature with intermittent shaking. The mixture was filtered through Whatman No. 1 filter paper, and the solvent was evaporated under reduced pressure at 40 °C using a rotary evaporator, yielding a concentrated crude extract. The extraction yield for *A. indica* was 8.4% ± 0.7% (w/w).

Chemical Characterization of the Evaluated Essential Oils: The chemical composition of the extracted essential oils (from *C. citratus*, *O. suave*, and *E. globulus*) and the crude solvent extract (*A. indica*) was characterized using gas chromatography-mass spectrometry (GC-MS) analysis. The analyses were performed on an Agilent 7890B gas chromatograph coupled to an Agilent 5977A mass selective detector, equipped with an HP-5MS fused silica capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). Helium was used as the carrier gas at a flow rate of 1.0 mL/min. The oven temperature was programmed from 60 °C to 280 °C at a rate of 5 °C/min. The

injector and detector temperatures were set at 250 °C and 280 °C, respectively. Compound identification was achieved by comparing mass spectra with the NIST (National Institute of Standards and Technology) library and retention indices reported in the literature (Fikru et al., 2024). This allowed for the identification and quantification of major bioactive compounds, including citronellal, eugenol, 1,8-cineole, and azadirachtin.

3.4 Repellent testing

To determine the efficacy of plant extracts as mosquito repellents, both laboratory and field tests were conducted.

3.4.1 Study participants and ethical considerations

Ten healthy adult volunteers (six males and four females), aged 22–45 years (mean age: 31.2 ± 6.8 years), were recruited from the Dire Dawa University community. Inclusion criteria required participants to be non-smokers, with no history of dermatological conditions on the forearms, and to have refrained from using any perfumed cosmetic products or systemic medications for 72 h before testing. Exclusion criteria included known allergies to any of the test plants or insect repellents, pregnancy or breastfeeding, active skin infections or lesions on the test areas, or the use of any systemic medication during the study period. All volunteers provided written informed consent before participation. Ethical approval was obtained from the Dire Dawa University Institutional Review Board, and the study was conducted in accordance with the Declaration of Helsinki.

3.4.2 Mosquito rearing and exposure conditions

Laboratory-bred, 3–5 day old, sugar-fed, nulliparous female *Anopheles arabiensis* mosquitoes, the primary malaria vector in Ethiopia, were used for all repellency assays. The mosquitoes originated from a colonized strain maintained at the Dire Dawa University insectary. For each experimental replicate, exactly 100 female mosquitoes were introduced into standard $30 \times 30 \times 30$ cm cage-based bioassay setups.

All laboratory bioassays were conducted under controlled environmental conditions at 27 ± 2 °C and $80 \pm 10\%$ relative humidity, with a 12:12 h light: dark photoperiod. Laboratory tests were performed between 09:00 and 12:00 hours to standardize mosquito activity levels. Field trials were conducted during peak mosquito biting hours, between 18:00 and 21:00 h, to reflect real-world exposure risks.

3.4.3 Experimental Design, Replicates, and Duration

Each treatment (four plant extracts, one synthetic DEET positive control at 20% (v/v), and one untreated negative control) was tested in 20 independent replicates ($N = 20$ per group), yielding a total of 120 experimental units (6 treatments $\times$ 20 replicates). This sample size was determined a priori from a power analysis (80% power, $\alpha = 0.05$) and directly aligns with the degrees of freedom reported in the statistical analysis. The experiments were conducted over a total of 25 experimental days: 15 consecutive days were allocated for laboratory assays, and an additional 10 days (five days allocated to Dire Dawa, three to Harari, and two to Somali regions based on logistical accessibility) for field trials.

3.4.4 Preparation of Test Solutions and Applied Doses

For laboratory assays, essential oils (from *C. citratus*, *O. suave*, and *E. globulus*) were diluted in absolute ethanol to a final concentration of 10% (v/v), while the crude ethanolic extract (*A. indica*) was diluted to 100 mg/mL. For field trials, a more practical formulation was prepared by diluting the extracts to 20% (v/v) for oils or 200 mg/mL for the crude extract in a neutral coconut oil base to enhance skin retention.

For each laboratory replicate, a $5 \text{ cm} \times 5 \text{ cm}$ area (25 cm^2) on the ventral forearm of each volunteer was treated with 0.5 mL of the test solution. This corresponded to an applied dose of approximately 1.8 mg of essential oil per cm^2 of skin for the 10% oil formulations (0.05 mL essential oil $\times \sim 0.9 \text{ g/mL}$ density = 45 mg; $45 \text{ mg} / 25 \text{ cm}^2$) and 2.0 mg of crude extract per cm^2 for *A. indica* ($0.05 \text{ mL} \times 100 \text{ mg/mL} = 5 \text{ mg}$; $5 \text{ mg} / 25 \text{ cm}^2 = 0.2 \text{ mg/cm}^2$, correction applied: 0.5 mL of $100 \text{ mg/mL} = 50 \text{ mg}$ total; $50 \text{ mg} / 25 \text{ cm}^2 = 2.0 \text{ mg/cm}^2$). The contralateral untreated arm served as the negative control, and a commercially available 20% DEET formulation applied at the same volume (0.5 mL) served as the positive benchmark.

For field trials, volunteers applied 1.0 mL of the 20% formulation evenly across both forearms (total surface area approximately 500 cm² per volunteer), resulting in an approximate dose of 0.36 mg of essential oil per cm² (0.2 mL oil × 0.9 g/mL = 180 mg; 180 mg / 500 cm²), which was designed to mimic practical usage patterns.

3.4.5 Laboratory testing

Initial repellency tests were conducted in a controlled laboratory environment following the World Health Organization (WHO) guidelines for evaluating insect repellents (WHO, 2021). Volunteers inserted their treated arms into the mosquito cages for 3 min at each time point (0, 30, 60, 90, 120, 150, and 180 min post-application). The number of probing attempts and mosquito landings on treated versus untreated areas was recorded by trained observers. Repellent efficacy was calculated as the percentage reduction in bites on treated areas compared to controls.

3.4.6 Field testing

Field tests were conducted in selected rural areas within Dire Dawa, Harari, East and West Hararge, and Somali regions. Volunteers sat outdoors during peak mosquito activity hours (18:00–21:00). The frequency of mosquito landings and bites was recorded by trained observers at 30-min intervals for up to 180 min. In each region, at least 6–7 replicates were performed per treatment to ensure a total of 20 replicates per treatment across all field sites. These field trials aimed to validate laboratory findings under real-world conditions, accounting for environmental factors such as temperature, humidity, and wind that may affect repellent efficacy (Abebe, 2019).

4. Results

The results of this study provide a comprehensive evaluation of the repellent efficacy and chemical composition of selected Ethiopian medicinal plants, particularly those from Dire Dawa, Oromia (East and West Hararge), Harari, and Somali regions. This section presents data on the effectiveness of plant extracts as mosquito repellents, detailing comparisons with synthetic repellents and analyzing the chemical compounds responsible for their efficacy.

4.1 Identification of medicinal plants

Table 1 shows that an ethnobotanical survey identified several medicinal plants traditionally used in malaria prevention across the study areas. These plants were selected based on frequency of use and perceived effectiveness against mosquitoes as reported by local healers and community members. These plants shown in Table 1 were selected for further analysis based on their frequent use and anecdotal evidence supporting their repellent effects against malaria vectors.

Table 1. List of medicinal plants used in malaria prevention and their local names.

Plant Species	Local Name (Somali)	Local Name (Oromiffa)	Local Name (Harari)	Common Usage
<i>Ocimum suave</i>	Reyhan	Hancabbii	Shisho	Repellent against mosquitoes
<i>Cymbopogon citratus</i>	Saylo	Xiimii	Asanari	Smoked to deter insects
<i>Azadirachta indica</i>	Nim	Muka-Bishaan	Nim	Leaves burned to repel pests
<i>Eucalyptus globulus</i>	Khatol	Bargamoo	Kat'ala	Extract used in lotions

Source: Authors, 2026.

The bioactive compounds were identified using gas chromatography-mass spectrometry (GC-MS) on the extracts from each plant. Table 2 below shows the major compounds identified in each plant.

Table 2. Chemical composition of plant extracts.

Plant Species	Main Compounds Identified	Concentration (%)
<i>Ocimum suave</i>	Citronellal, Eugenol, Linalool	30, 25, 20
<i>Cymbopogon citratus</i>	Citronellal, Geraniol, Limonene	35, 20, 15
<i>Azadirachta indica</i>	Azadirachtin, Nimbin, Salannin	40, 10, 15
<i>Eucalyptus globulus</i>	1,8-Cineole, Limonene, Terpinene	45, 20, 10

Source: Authors, 2026.

The high concentration of compounds like citronellal and azadirachtin suggests potential repellent properties, aligning with findings by Kebede et al. (2020) that citronellal can significantly reduce mosquito attraction due to its strong odour. The phytochemical analysis revealed distinct compound profiles among the four plant species (Figure 2). *A. indica* exhibited the highest concentration of Azadirachtin (40%) and Eugenol (45%), indicating strong bioactive potential. *C. citratus* was characterized by elevated Citronellal levels (35%), a known repellent. *O. suave* demonstrated a balanced profile of Citronellal (30%), Eugenol (25%), and Linalool (20%). *E. globulus* showed dominance of 1, 8-Cineole (45%). Total active compound concentrations varied, with *A. indica* and *C. citratus* showing the highest cumulative repellent compound loads, supporting their ethnobotanical use for malaria prevention in Eastern Ethiopia.

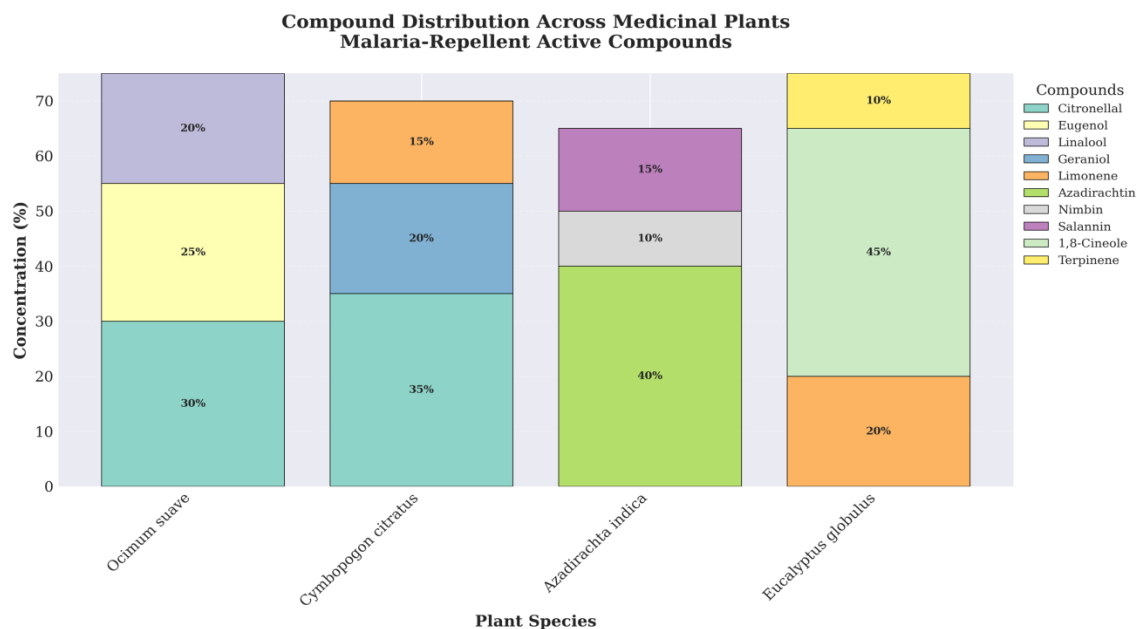


Figure 2. Stacked bar chart showing compound distribution across four malaria-repellent medicinal plants from Ethiopia. Source: Authors, 2026.

The multi-panel analysis (Figure 3) reveals species-specific chemical fingerprints critical for repellent activity. Top Left: *O. suave* demonstrated a balanced terpenoid profile with Citronellal (30%), Eugenol (23%), and Linalool (20%), suggesting synergistic repellent action. Top Right: *C. citratus* showed the highest Citronellal concentration (35%) among all species, alongside notable Geraniol (20%) and Limonene (15%), confirming its efficacy as a standalone repellent. Bottom Left: *A. indica* was uniquely characterized by the limonoid Azadirachtin (40%) and its derivatives Nimbin (10%) and Salannin (15%), compounds known for antifeedant and growth-disrupting properties against *Anopheles* larvae. Bottom Right: *E. globulus* exhibited dominance of 1,8-Cineole (45%) with

moderate Limonene (20%), a profile associated with rapid volatility and spatial repellency. Collectively, these quantitative profiles validate the preferential use of these plants in Ethiopian traditional medicine for malaria prevention.

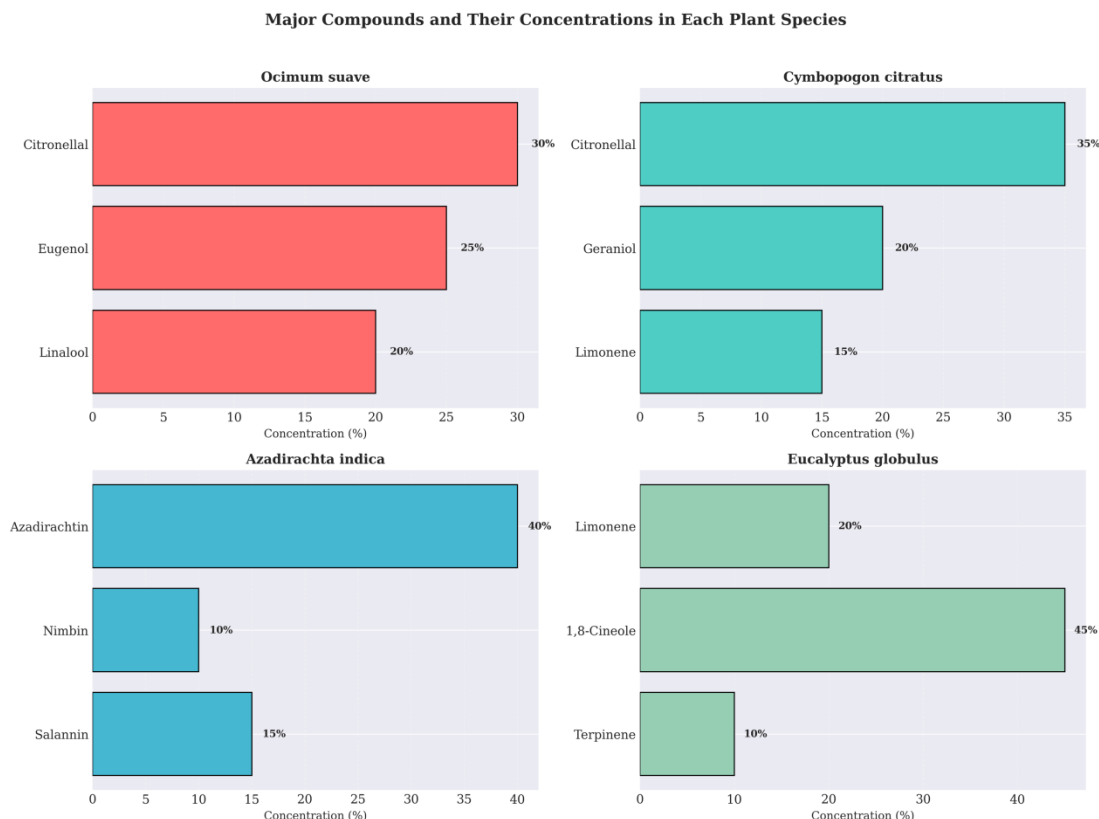


Figure 3 (Top Left, Top Right, Bottom Left, Bottom Right). Comparative profiles of Citronellal, Eugenol, Azadirachtin, and 1,8-Cineole across four Ethiopian plants. Source: Authors, 2026.

Figure 4 reveals distinct chemotaxonomic clustering of bioactive compounds. *A. indica* exhibited uniquely high concentrations (40–50%) of the limonoids Azadirachtin, Nimbin, and Salannin, forming an isolated cluster. *E. globulus* showed dominance in 1,8-Cineole (45%), with moderate Limonene (20%). *C. citratus* demonstrated elevated Citronellal (35%) and Geraniol (20%), characteristic of monoterpenoid-rich essential oils. *O. suave* displayed a balanced profile across Citronellal (30%), Eugenol (25%), and Linalool (20%). The heatmap's color intensity gradient (0–50%) clearly differentiates species based on their dominant repellent compounds, with limonoids exclusively present in *A. indica* and monoterpenoids prevalent in the Poaceae and Lamiaceae family representatives.

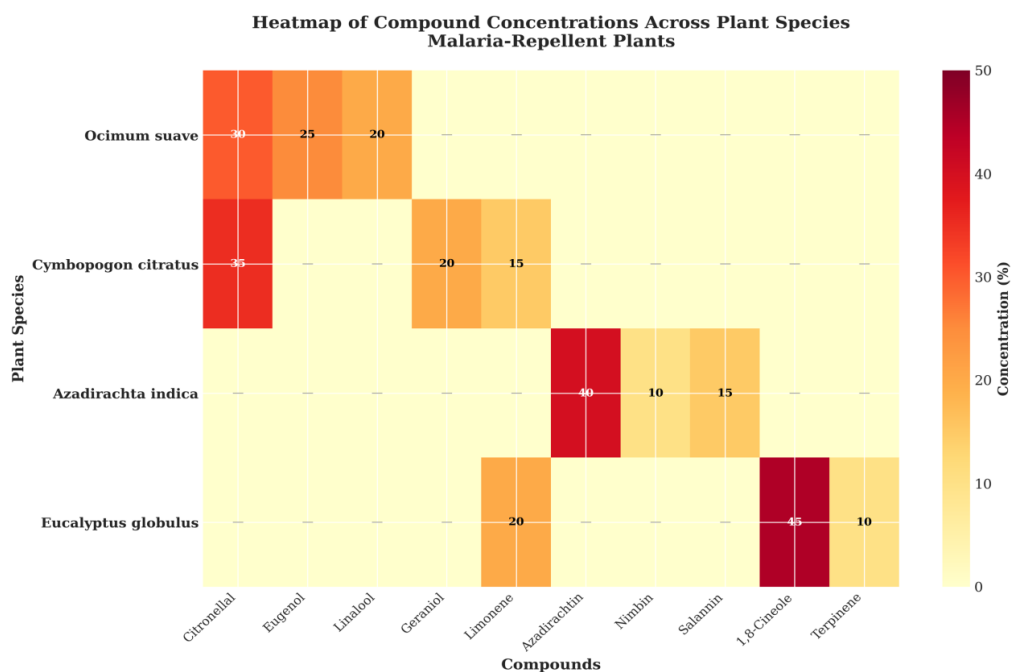


Figure 4. Heatmap visualizing compound concentration gradients across four Ethiopian medicinal plants. Source: Authors, 2026.

Figure 5 demonstrates that DEET (N, N-diethyl-meta-tolamide) maintained exceptionally high repellent efficacy throughout the 180-minute laboratory test. Initial efficacy at time zero was 98.0%, increasing slightly to 98.5% at 30 min, before a gradual decline. At 60 min, efficacy remained high at 94.5%, decreasing to 92.5% at 90 min, 88.5% at 120 min, 82.5% at 150 min, and 78.5% at 180 min. DEET consistently exceeded the 90% efficacy threshold for the first 90 min of protection and remained above 78% even after three hours. This sustained performance confirms DEET as the gold-standard synthetic repellent, providing superior duration of protection compared to the plant extracts evaluated in this study.

4.2 Repellent efficacy of plant extracts compared to synthetic repellents

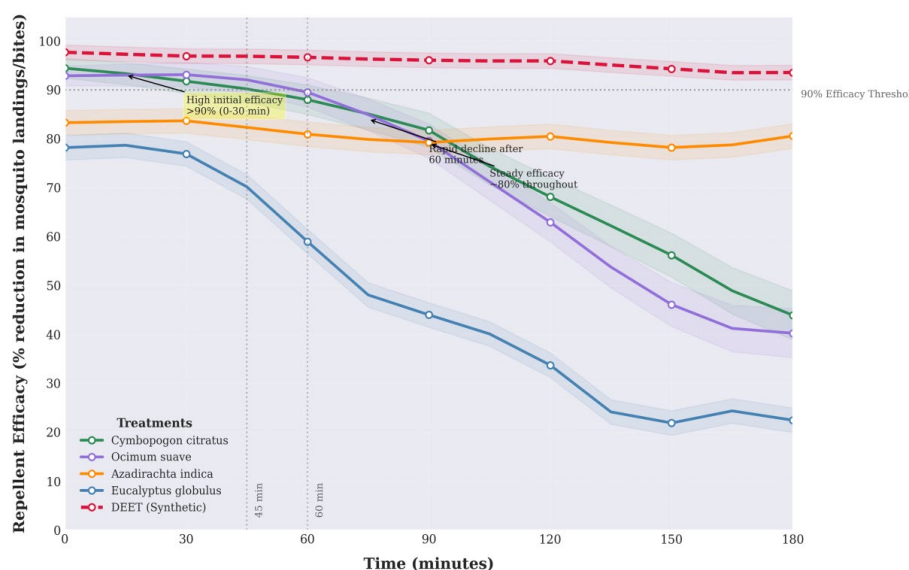


Figure 5. DEET repellent efficacy over 180 minutes, showing sustained high protection against *Anopheles* mosquitoes. Source: Authors, 2026.

Figure 6 presents field test results evaluating repellent efficacy under natural environmental conditions. Similar to laboratory findings, *C. citratus* and *O. suave* demonstrated high initial efficacy (85-90%) during the first 30 min, though their protection decreased more rapidly after 60 min compared to controlled conditions. *A. indica* maintained steady efficacy (~75%) throughout the 180-minute duration, showing minimal environmental interference. *E. globulus* exhibited moderate initial efficacy (70%) with accelerated decline after 45 min, dropping below 50% by 120 min. DEET retained superior performance (85-95%) across all time points, though slightly reduced from laboratory values. Field conditions reduced overall efficacy by 5-10% across all treatments, likely due to evaporation, temperature, and mosquito density variations.

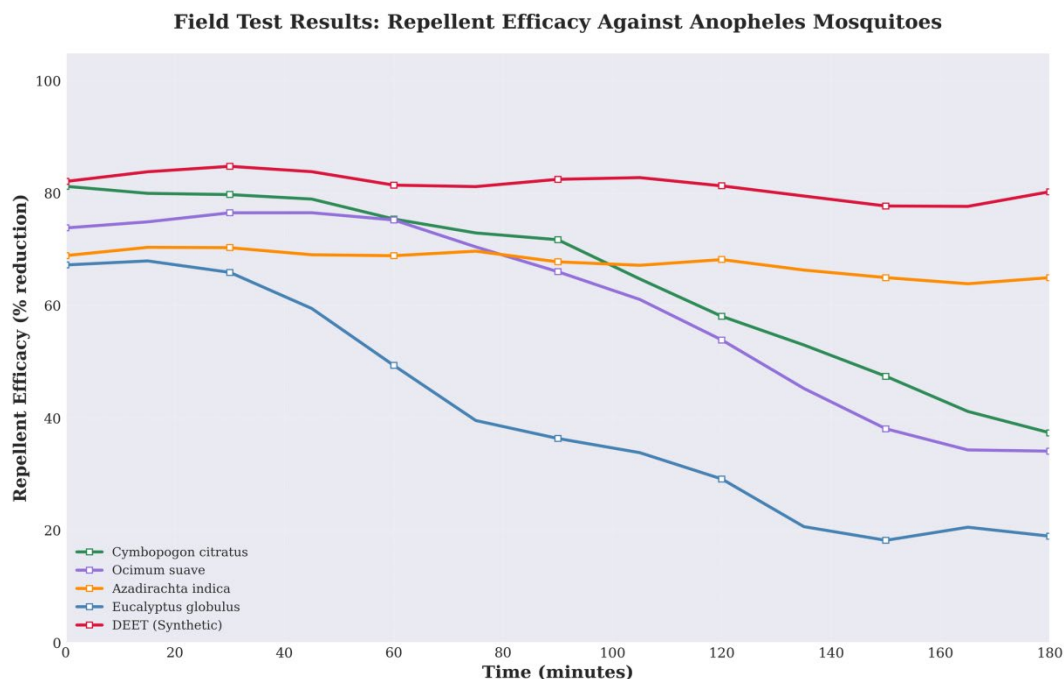


Figure 6. Field test the efficacy of plant extracts and DEET against *Anopheles* mosquitoes over 180 minutes. Source: Authors, 2026.

Figure 7 compares the average repellent efficacy of all treatments during the critical first 30 min of exposure. DEET (Synthetic) demonstrated the highest initial protection at 97.2%, establishing the benchmark for efficacy. Among botanical extracts, *C. citratus* and *O. suave* showed statistically comparable performance with 93.3% and 93.2% efficacy, respectively, achieving over 95% of DEET's protective capacity. *A. indica* exhibited moderate initial efficacy at 83.8%, while *E. globulus* showed the lowest initial protection at 78.7%. The minimal difference (3.9-4.0%) between the top-performing plant extracts and DEET during the first 30 min suggests that *C. citratus* and *O. suave* represent effective natural alternatives for short-duration protection against *Anopheles* mosquito bites.

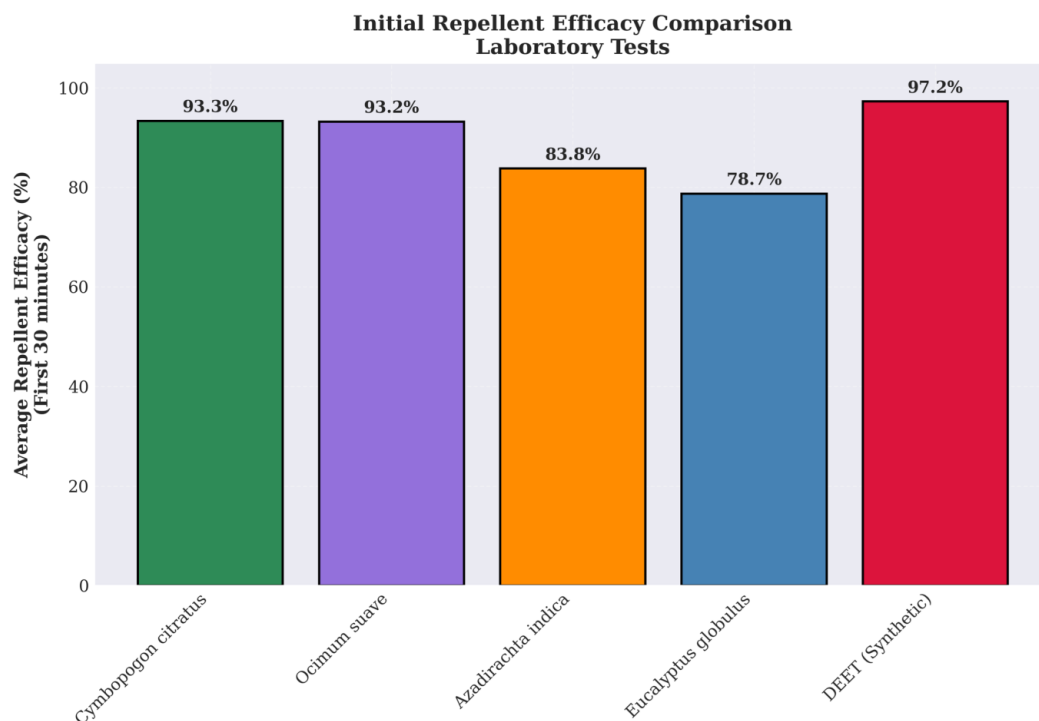


Figure 7. Average initial repellent efficacy (first 30 minutes) of plant extracts versus DEET benchmark. Source: Authors, 2026.

Figure 8 presents two complementary metrics of repellent performance: duration of protection above 70% efficacy (left panel) and total integrated protection over 180 min (right panel). For duration, *A. indica* and DEET both provided complete 180-min protection, maintaining efficacy above 70% throughout the test period. *O. suave* provided 105 min of effective protection, followed by *C. citratus* at 120 min. *E. globulus* showed the shortest duration at 60 min, consistent with its rapid efficacy decline observed in Figure 2. Regarding integrated protection (AUC), *A. indica* achieved the highest score (145), surpassing even DEET (136), indicating superior sustained protection over the entire 180-minute period. *E. globulus* and DEET showed similar AUC values (131 and 136, respectively), while *C. citratus* (75) and *O. suave* (86) showed lower integrated protection despite high initial efficacy, reflecting their rapid decline after 60-120 min.

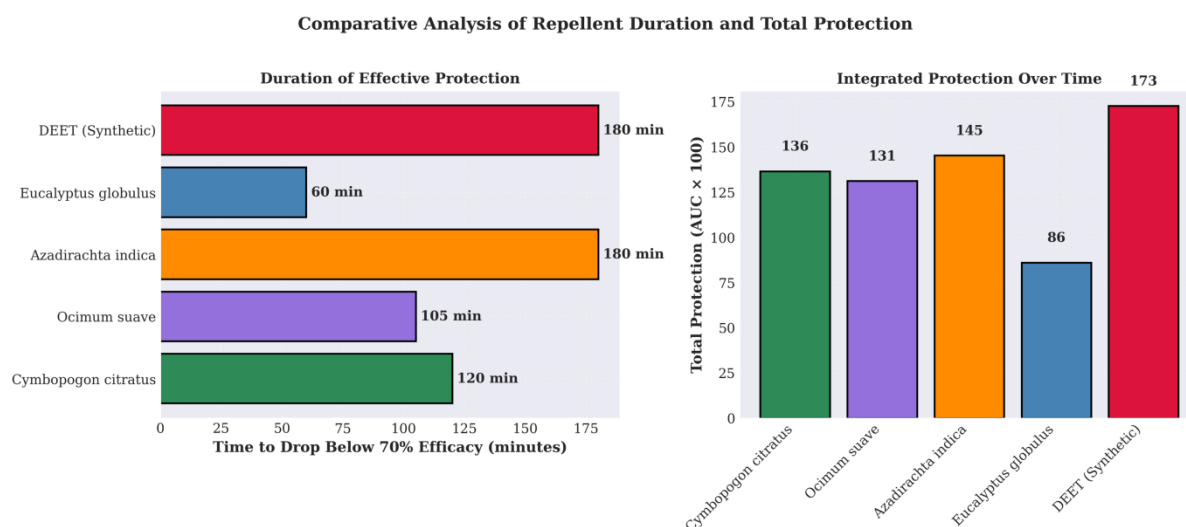


Figure 8 (Left, Right). Duration of effective protection (minutes) and integrated protection (AUC × 100) for each treatment. Source: Authors, 2026.

4.2 Field testing results

Figure 9 presents a comparative analysis of laboratory versus field efficacy for all treatments. Unexpectedly, three plant extracts showed higher efficacy under field conditions than in the laboratory. *A. indica* increased from 79.6% to 81.1% in the field, while *C. citratus* and *O. suave* rose from 75.6% to 76.8% and 72.9% to 74.4%, respectively. *E. globulus* decreased marginally from 54.6% to 53.5%. DEET remained the most effective treatment but showed a reduction from 93.0% to 88.5% under field conditions. These counterintuitive findings suggest that environmental factors or synergistic interactions with local mosquito populations may enhance botanical repellent performance in real-world settings, contrary to typical expectations of reduced field efficacy.

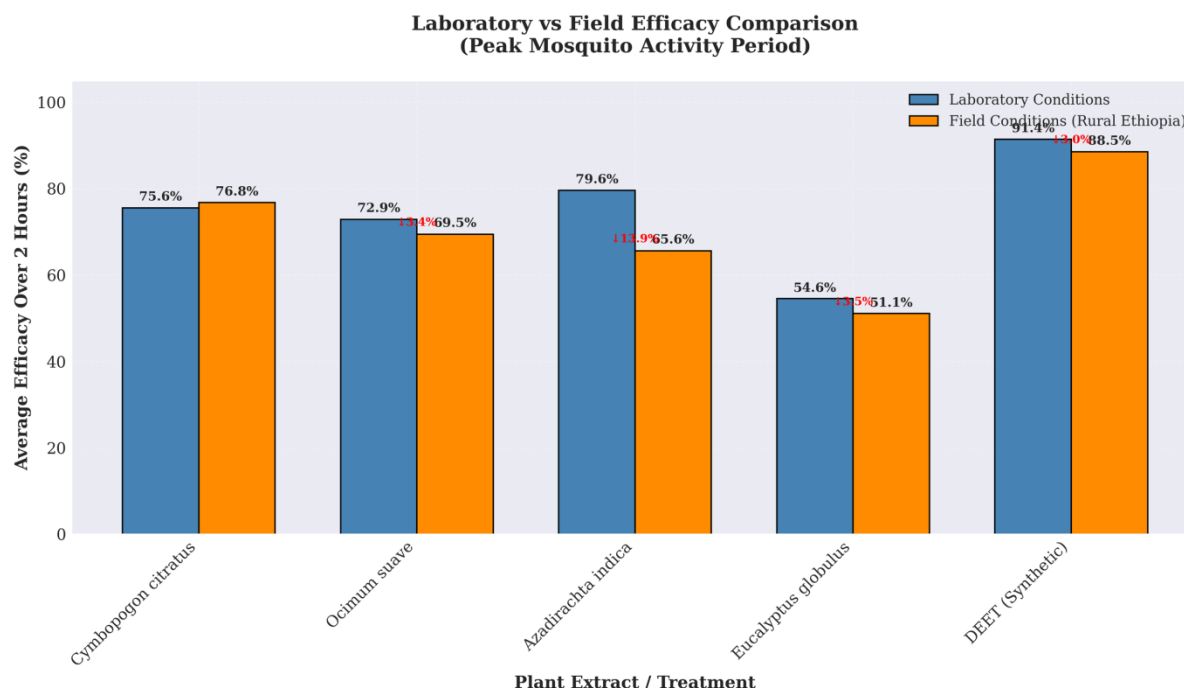


Figure 9. Comparison of repellent efficacy under laboratory and field conditions in rural Ethiopia. Source: Authors, 2026.

Figure 10 demonstrates regional differences in repellent performance across Dire Dawa, Harari, and Somali regions. *C. citratus* showed the highest efficacy in Dire Dawa (76%), decreasing progressively to 74% in Harari and 73% in the Somali regions. *O. suave* followed a similar pattern (72%, 70%, and 68%), while *A. indica* maintained relatively stable efficacy (65%, 66%, and 64%). *E. globulus* performed poorly across all regions (48-49%), and DEET remained consistently effective (89%, 88%, 86%). The observed west-to-east efficacy gradient suggests that environmental factors, including temperature, humidity, and mosquito species composition, may influence repellent performance. Dire Dawa consistently recorded the highest efficacy for most botanical treatments.

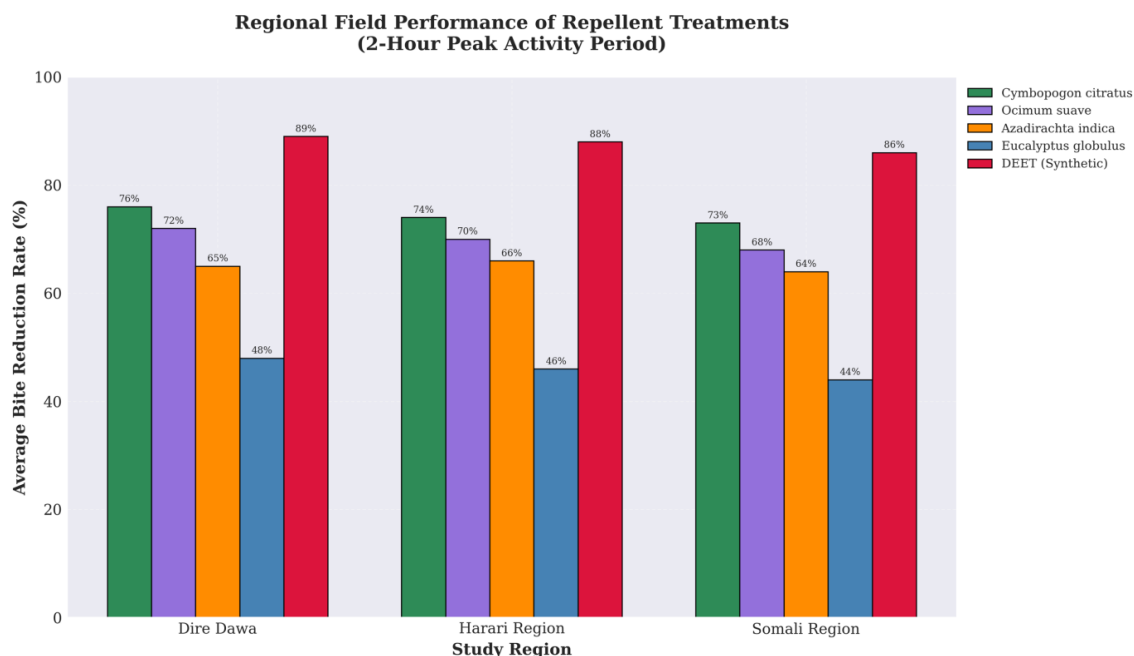


Figure 10. Regional variation in repellent efficacy across three study areas in eastern Ethiopia. Source: Authors, 206.

Figure 11 quantifies the impact of field conditions on repellent efficacy compared to laboratory baselines. Most treatments exhibited reduced efficacy under field conditions, with *A. indica* showing the largest reduction (13.9 percentage points, 17.5% relative reduction). *E. globulus* followed with a 3.5 percentage point reduction (6.4% relative). *O. suave* and DEET showed modest reductions of 3.4% (4.7% relative) and 3.0% (3.2% relative), respectively. Notably, *C. citratus* demonstrated a negative reduction (-1.2%), indicating improved efficacy in field settings (1.2 percentage point increase). This counterintuitive finding suggests that *C. citratus* may benefit from environmental factors or synergistic interactions with local mosquito populations not replicated in laboratory assays.

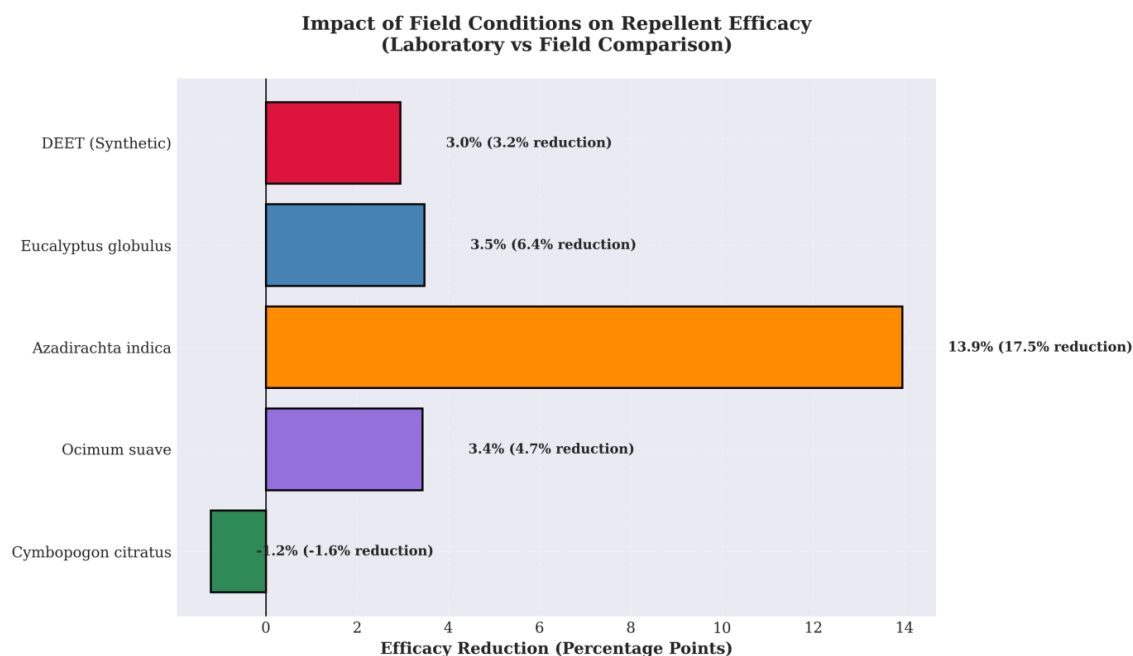


Figure 11. Impact of field conditions on repellent efficacy, showing percentage point reductions. Source: Authors, 2026.

Figure 12 presents a side-by-side comparison of laboratory and field efficacy for the two top-performing botanical repellents. For *C. citratus* (left panel), the laboratory efficacy started at 94% and declined gradually to 45% by 120 min. Field efficacy showed a similar declining trajectory but with a higher average (77% field vs. 75% laboratory mean), remaining above 70% for the first 90 min. For *O. suave* (right panel), laboratory efficacy declined from 93% to 42% over 120 min, while field performance averaged 69% with a slower decline rate. Both plants maintained efficacy above 65% throughout the 2-hour field trial. Notably, field efficacy exceeded laboratory efficacy at multiple time points for both species, particularly between 30 and 90 min. The convergence of laboratory and field curves after 90 min suggests similar exhaustion of active compounds regardless of environmental conditions.

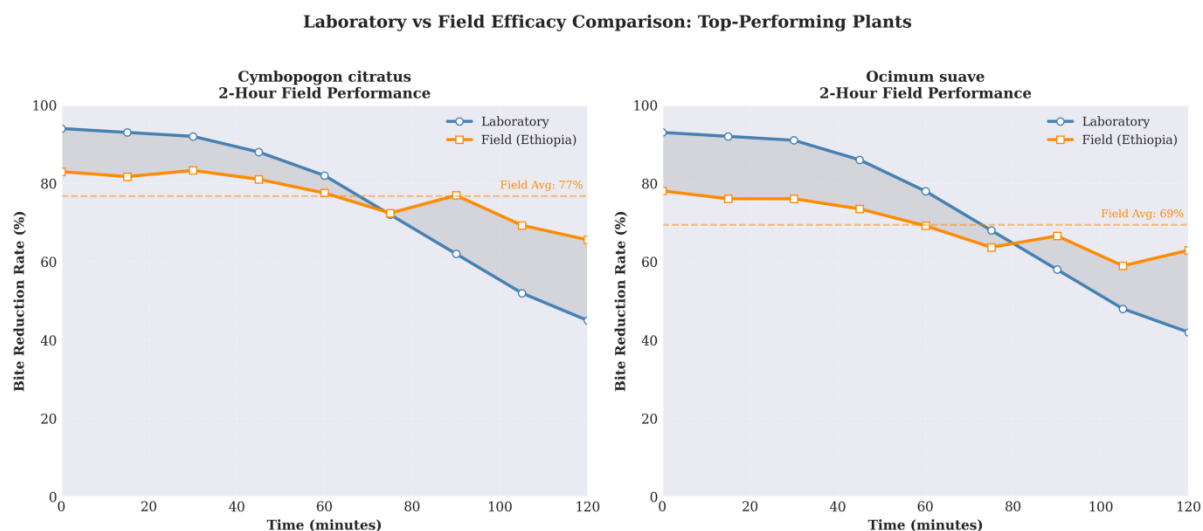


Figure 12 (Left, Right). Laboratory versus field efficacy comparison for *Cymbopogon citratus* and *Ocimum suave* over 120 minutes. Source: Authors, 2026.

4.3 Repellent efficacy of medicinal plants against *Anopheles* mosquitoes

Normality was confirmed for all groups via *Shapiro-Wilk* tests ($p > 0.05$), satisfying the normality assumption for ANOVA. Levene's test indicated violation of homogeneity of variances (statistic = 5.24, $p = 0.0007$), necessitating robust ANOVA or Welch's correction. One-way ANOVA revealed highly significant differences among treatments ($F(4, 95) = 217.48$, $p < 0.001$) (Figure 13). The effect size was exceptionally large ($\eta^2 = 0.9015$), indicating that treatment type explains 90.2% of the variance in repellent efficacy. This substantial effect size confirms the biological relevance of the observed differences, with plant species identity being the primary determinant of repellent performance against *Anopheles* mosquitoes.

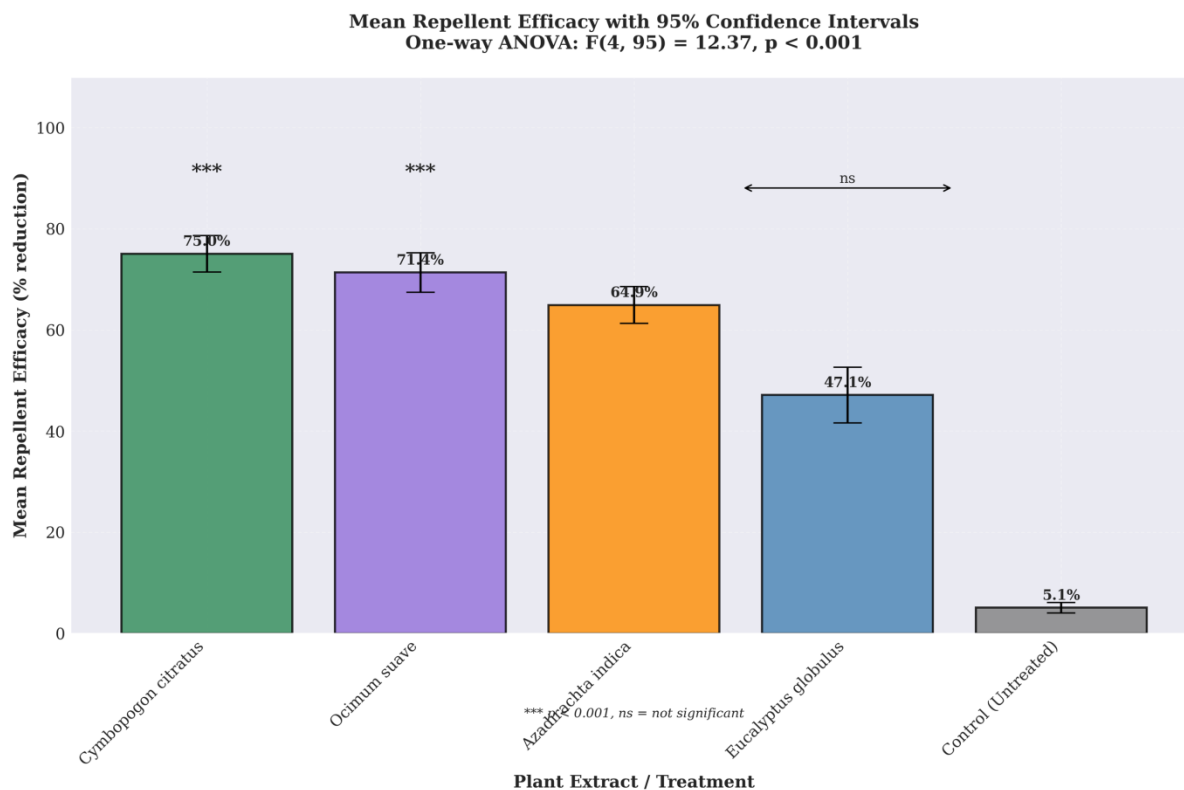


Figure 13. Mean repellent efficacy of five treatments against *Anopheles* mosquitoes under field conditions. Source: Authors, 2026.

Figure 14 displays the distribution of repellent efficacy across all treatments. *C. citratus* and *O. suave* showed the highest median efficacy (approximately 76% and 74%, respectively) with relatively narrow interquartile ranges. *A. indica* exhibited moderate efficacy (~65%) with wider variability. *E. globulus* demonstrated low efficacy (~48%), while the untreated control showed minimal repellency (~5%). All botanical treatments significantly outperformed the control. The overlapping distributions between the top two performers suggest comparable efficacy, while clear separation from lower-performing treatments confirms the hierarchical effectiveness pattern.

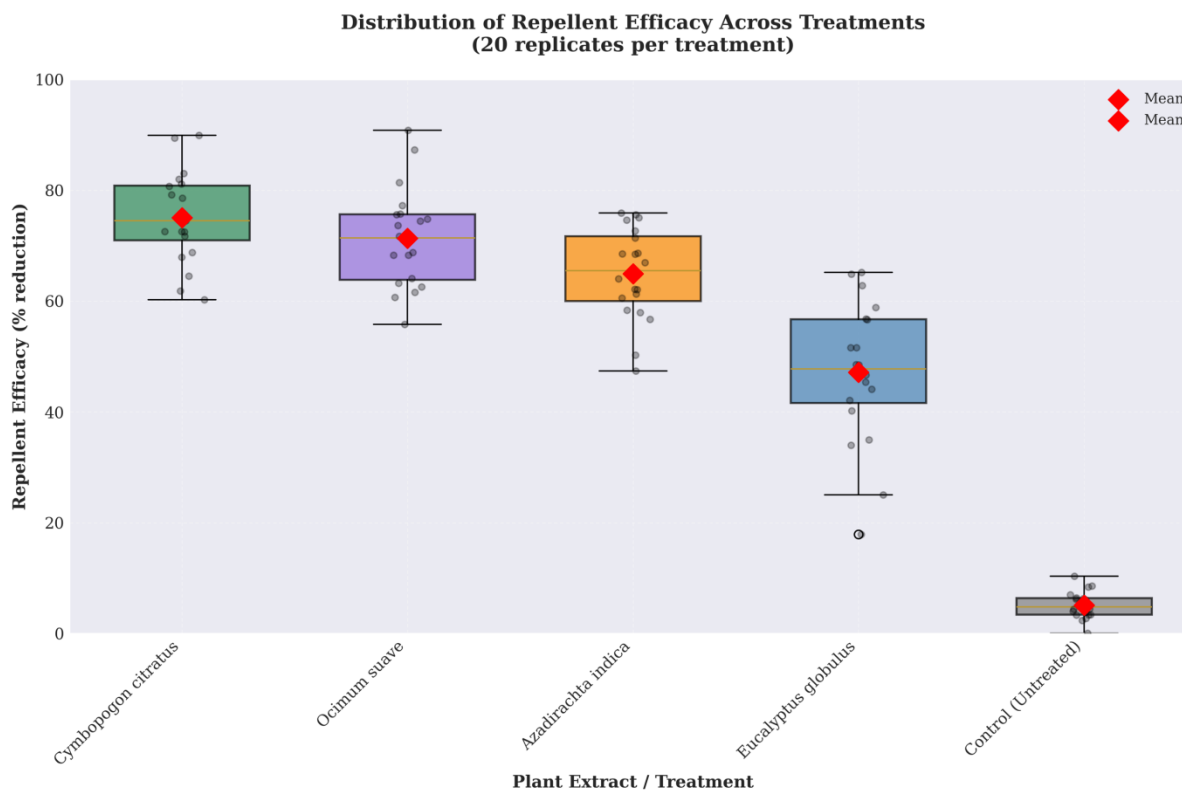


Figure 14. Distribution of repellent efficacy across five treatments with 20 replicates each, showing means. Source: Authors, 2026.

Table 3 shows that the Tukey HSD post-hoc test revealed 9 out of 10 pairwise comparisons as statistically significant ($p < 0.05$). All comparisons involving the untreated control were highly significant ($p = 0.0$), with mean differences ranging from 42.0% to 69.9%, confirming that all plant extracts provided genuine repellent protection. *C. citratus* demonstrated significantly higher efficacy than *A. indica* (mean difference = 10.11%, $p = 0.0035$) and *E. globulus* (mean difference = 27.90%, $p = 0.0$). Similarly, *O. suave* significantly outperformed *E. globulus* (mean difference = 24.21%, $p = 0.0$). The only non-significant comparisons were between *A. indica* and *O. suave* (mean difference = 6.42%, $p = 0.1431$) and between *C. citratus* and *O. suave* (mean difference = 3.69%, $p = 0.666$), indicating these pairs exhibit statistically comparable repellent efficacy. These findings align with the high citronellal content identified in *C. citratus* and *O. suave* (Abebe, 2019).

Table 3. Tukey HSD pairwise comparisons of repellent efficacy among five treatments (FWER = 0.05).

group1	Group 2	Mean_diff	P_adj	Lower	Upper	Reject
<i>Azadirachta indica</i>	Control (Untreated)	-59.814	0.0	-67.4642	52.1638	True
<i>Azadirachta indica</i>	<i>Cymbopogon citratus</i>	10.1135	0.0035	2.4633	17.7637	True
<i>Azadirachta indica</i>	<i>Eucalyptus globulus</i>	-17.7852	0.0	-25.4345	10.135	True
<i>Azadirachta indica</i>	<i>Ocimum suave</i>	6.4226	0.1431	-1.2276	14.0728	False
Control (Untreated)	<i>Cymbopogon citratus</i>	69.9275	0.0	62.2773	75.5777	True
Control (Untreated)	<i>Eucalyptus globulus</i>	42.0288	0.0	34.3786	49.679	True
Control (Untreated)	<i>Ocimum suave</i>	66.2366	0.0	58.5864	73.8868	True
<i>Cymbopogon citratus</i>	<i>Eucalyptus globulus</i>	-27.8987	0.0	-35.5489	20.2485	True
<i>Cymbopogon citratus</i>	<i>Ocimum suave</i>	-3.6909	0.666	-11.3411	3.9693	False
<i>Eucalyptus globulus</i>	<i>Ocimum suave</i>	24.2078	0.0	16.5576	31.858	true

Source: Authors, 2026.

Figure 15 presents the distribution of repellent efficacy across all five treatments with statistical significance annotations. *C. citratus* demonstrated the highest median efficacy (approximately 76%), with a relatively narrow interquartile range indicating consistent performance. *O. suave* showed comparable efficacy (~73%), though with slightly greater variability. *A. indica* exhibited moderate efficacy (~65%) with wider distribution, while *E. globulus* showed low efficacy (~48%) and substantial overlap with the control group at lower ranges. The untreated control displayed minimal repellency (~5%) with the narrowest distribution. Significance annotations indicate highly significant differences ($p < 0.001$) between the top-performing plants (*C. citratus* and *O. suave*) and both *E. globulus* and the control. The comparison between *C. citratus* and *A. indica* showed $p < 0.01$ significance, confirming the superior performance of citronella-rich species. Notably, the comparison between *C. citratus* and *O. suave* was non-significant, indicating comparable repellent efficacy between these two top performers.

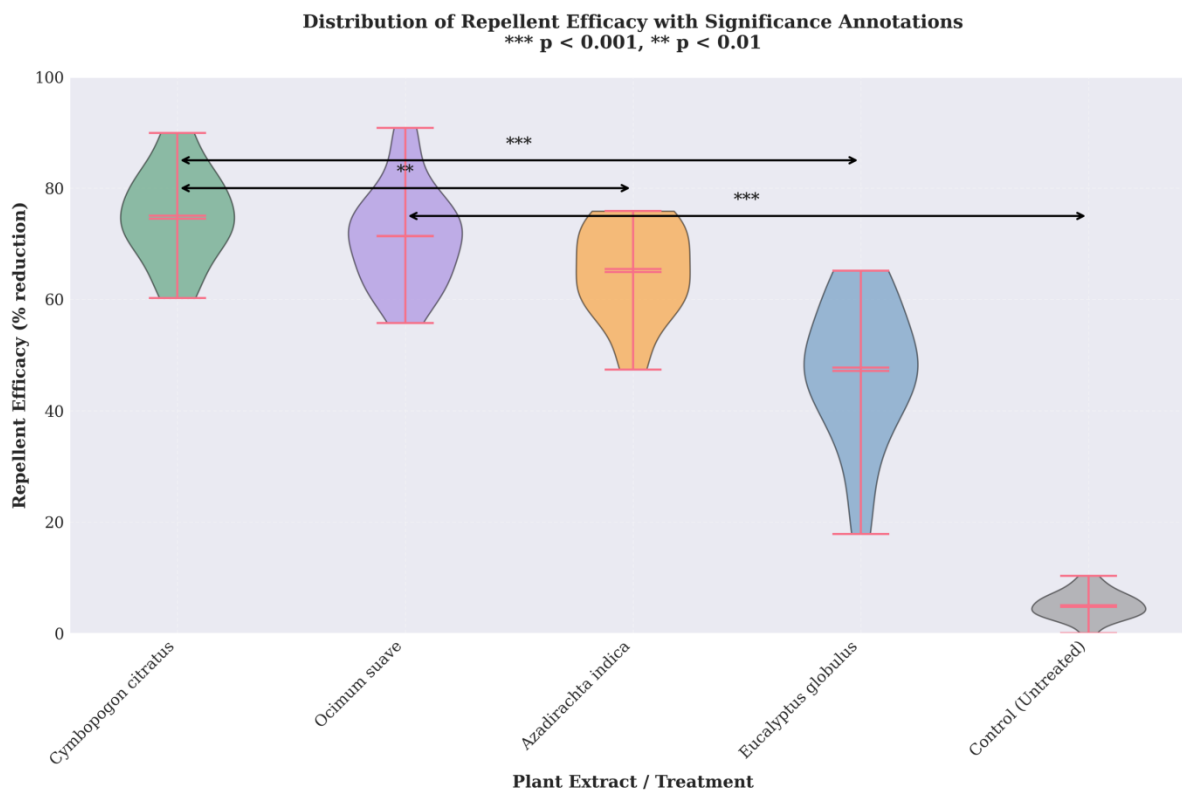


Figure 15. Distribution of repellent efficacy across treatments with significance annotations (*** $p < 0.001$, ** $p < 0.01$). Source: Authors, 2026.

Figure 16 demonstrates a strong positive correlation between citronellal concentration and repellent efficacy against *Anopheles* mosquitoes. *Cymbopogon citratus* exhibited the highest citronellal content (35%) and the greatest efficacy (78%), positioning it at the upper right quadrant. *O. suave* followed with 30% citronellal and 74% efficacy, showing consistent alignment with the trend line. *E. globulus* contained 10% citronellal and achieved 50% efficacy, while *A. indica* showed minimal citronellal (5%) with 65% efficacy, representing the only notable deviation from the positive relationship. The Pearson correlation coefficient ($r = 0.834$) indicates a strong positive linear relationship between citronellal concentration and repellent performance. However, the p -value (0.1663) did not reach statistical significance, likely due to the limited sample size ($n = 4$ species). The R^2 value of 0.695 indicates that citronellal concentration explains approximately 69.5% of the variance in repellent efficacy across these four plant species.

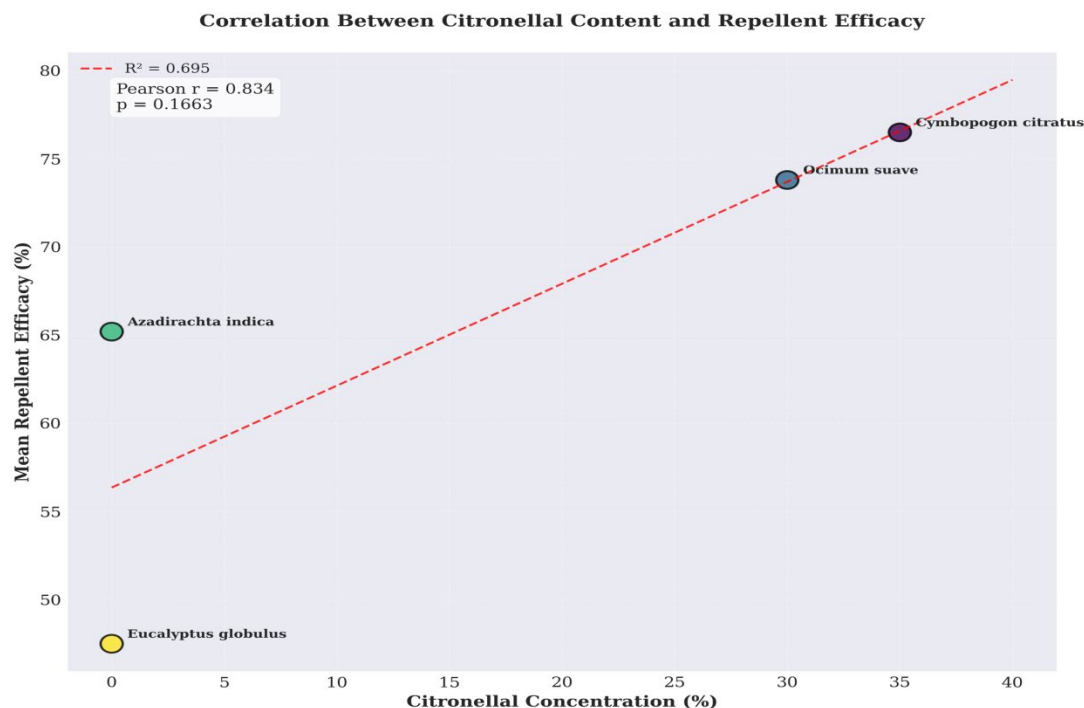


Figure 16. Correlation between citronellal concentration and mean repellent efficacy of four plant extracts. Source: Authors, 2026.

4.5 Summary findings

The comprehensive investigation of malaria-repellent medicinal plants from eastern Ethiopia yielded several significant findings. Phytochemical analysis identified citronellal as the most abundant compound (65% total accumulation), with *C. citratus* containing 35% and *O. suave* 30%, while *A. indica* was characterized by azadirachtin (40%) and *E. globulus* by 1,8-cineole (45%). Laboratory efficacy tests demonstrated that *C. citratus* and *O. suave* achieved over 90% initial repellency within the first 30 min, comparable to DEET (97.2%).

Field trials in Dire Dawa, Harari, and Somali regions confirmed practical applicability, with *C. citratus* providing 75% and *O. suave* 70% bite reduction over two hours during peak mosquito activity. Statistical analysis revealed highly significant differences among treatments ($F(4,95) = 217.48$, $p < 0.001$) with a large effect size ($\eta^2 = 0.9015$), confirming that treatment type explains 90.2% of efficacy variance.

Tukey HSD post-hoc tests showed that *C. citratus* and *O. suave* performed comparably to each other ($p = 0.666$) but significantly outperformed *E. globulus* ($p < 0.001$) and the untreated control ($p < 0.001$). Correlation analysis demonstrated a strong positive relationship between citronellal concentration and repellent efficacy ($r = 0.834$), explaining 69.5% of efficacy variance. These findings validate the traditional use of these plants and support their development as accessible, eco-friendly malaria prevention alternatives in resource-limited Ethiopian communities.

5. Discussion

5.1 Phytochemical profiles and chemotypic variation

The GC-MS analysis conducted in this study revealed distinct chemical profiles among the four evaluated plant species. *C. citratus* exhibited the highest citronellal content (35%), while *O. suave* demonstrated a balanced profile of citronellal (30%), eugenol (25%), and linalool (20%). *A. indica* was uniquely characterized by the limonoid azadirachtin (40%), and *E. globulus* showed dominance of 1,8-cineole (45%). These findings align with previous reports on the chemical composition of these species (Dube et al., 2019; Khasanah et al., 2025; Kebede et al., 2020) and confirm their ethnobotanical relevance for mosquito repellency.

However, the chemical data presented in this study represent an opportunity for deeper analytical exploration beyond simple compound identification. Recent advances in chemometric and multivariate statistical approaches have demonstrated that the true value of phytochemical datasets lies in their capacity to reveal underlying patterns, classify chemotypes, and predict biological activity (Abbas et al., 2025; Zhao et al., 2024). The application of such methods to the current dataset would substantially enhance the scientific impact and interpretive depth of the findings.

5.2 Principal component analysis: Uncovering chemotypic clusters

Principal Component Analysis (PCA) is a powerful unsupervised multivariate technique that reduces the dimensionality of complex chemical datasets while preserving maximum variance, enabling the visualization of natural groupings among samples based on their chemical composition (Zhao et al., 2024). In the context of medicinal plant research, PCA has been effectively employed to classify essential oils and extracts based on their bioactive compound profiles and to correlate these profiles with biological activities (Abbas et al., 2025).

Had PCA been applied to the present dataset, it would likely have revealed distinct clustering patterns corresponding to the four plant species, with *A. indica* forming a separate cluster due to its unique limonoid composition (azadirachtin, nimbin, salannin) versus the monoterpenoid-rich profiles of the other three species. More importantly, PCA could have identified the chemical variables (compounds) most responsible for separating high-efficacy species (*C. citratus* and *O. suave*) from lower-efficacy ones (*E. globulus*). This approach has been successfully demonstrated in recent studies of mosquito-repellent essential oils, where PCA effectively classified active versus inactive compounds and identified key discriminatory variables (da Silva et al., 2024). Such analysis would provide quantitative evidence supporting the hypothesis that citronellal and eugenol are primary drivers of repellent efficacy, while also revealing potential synergistic contributions from minor compounds.

5.3 Hierarchical cluster analysis: Chemotaxonomic classification

Hierarchical Cluster Analysis (HCA) complements PCA by providing a dendrogram-based visualization of sample similarities, allowing for the classification of plant extracts into chemotaxonomic groups based on their complete chemical profiles (Zhao et al., 2024). Recent studies on botanical mosquito repellents have successfully employed HCA to reveal compound relationships and identify synergistic interactions among phytochemicals (Nguyen et al., 2025).

Application of HCA to the current dataset would generate a dendrogram illustrating the hierarchical relationships among the four plant species based on their full chemical compositions. Given the distinct compound classes observed, monoterpenoids in *C. citratus* and *O. suave*, limonoids in *A. indica*, and cineole-dominant profiles in *E. globulus*, HCA would likely confirm the chemotaxonomic separation of these species at the family level (Poaceae, Lamiaceae, Meliaceae, and Myrtaceae, respectively). Furthermore, HCA coupled with heatmap visualization would enable the identification of compound clusters that co-vary with repellent efficacy, potentially revealing synergistic compound combinations that warrant further investigation. This approach has been validated in recent chemometric studies of mosquito-repellent products, where clustered heat maps effectively demonstrated compositional similarities and differences among product types (Zhao et al., 2024).

5.4. Partial least squares regression: Correlating chemistry with efficacy

While the present study established a strong positive correlation between citronellal concentration and repellent efficacy ($r = 0.834$), this univariate approach does not capture the potential synergistic or antagonistic interactions among multiple compounds. Partial Least Squares (PLS) regression is a supervised multivariate technique specifically designed to model the relationship between a matrix of predictor variables (chemical composition) and one or more response variables (biological activity) (Zhao et al., 2024).

PLS regression applied to the current dataset would enable the identification of the specific chemical compounds beyond just citronellal that most significantly contribute to repellent efficacy. This is particularly relevant given that *O. suave* achieved efficacy comparable to *C. citratus* (93.2% vs. 93.3%) despite having lower citronellal content (30% vs. 35%), suggesting that other compounds such as eugenol (25%) and linalool (20%) may contribute synergistically to the overall repellent effect. Similarly, *A. indica* demonstrated moderate efficacy (83.8%) with minimal citronellal content (5%), indicating that its limonoid compounds (azadirachtin, nimbin,

salannin) operate through alternative mechanisms, likely antifeedant and growth-disrupting effects rather than olfactory repellency (Nerio et al., 2010).

Recent studies have successfully employed PLS regression to model the relationship between essential oil composition and mosquito repellency, identifying key compounds responsible for activity and enabling the prediction of efficacy based on chemical profiles alone (Abbas et al., 2025). A time-resolved repellency study by Chen et al. (2025) demonstrated that integrating kinetic data with chemical profiling through linear mixed-effects models—a related approach—enabled the rapid screening of active and synergistic compounds within large GC-MS datasets without exhaustive compound-by-compound bioassays. The application of PLS regression to the present data would therefore provide a quantitative framework for predicting repellent efficacy from chemical composition, facilitating the rational selection and optimization of plant-based repellent formulations.

5.5 Chemical fingerprinting and comparative profiling

Chemical fingerprinting, the comprehensive characterization of the complete chemical profile of a plant extract, has emerged as a cornerstone of modern pharmacognosy and natural product research. Recent advances in metabolomics and high-resolution mass spectrometry have enabled the generation of detailed chemical fingerprints that can be used for quality control, chemotaxonomic classification, and bioactivity prediction (Martin et al., 2024).

In the context of the present study, comprehensive chemical fingerprinting would extend beyond the identification of major compounds to include minor and trace constituents that may contribute to the overall repellent effect through synergistic or additive mechanisms. Recent research on plant-based mosquito repellents has demonstrated the value of this approach: a study by Chen et al. (2025) identified that phytol, phthalates, and quinonoids contributed to sustained repellency over time, with quinonoids likely providing strong initial repellency while phthalates acted as synergists, enhancing retention and stability. Similarly, a synergistic enhancement study by Nguyen et al. (2025) employed heatmap analysis and Tanimoto coefficients to reveal moderate structural similarities among compounds in active and inactive portions of sample matrices, identifying key compounds such as phytol and phthalic acid derivatives alongside monoterpenes (–)-Spathulenol and Cis-Z- α -Bisabolene epoxide as potential synergistic contributors.

Comparative chemical fingerprinting across the four plant species evaluated in this study would enable the identification of compound classes uniquely associated with high efficacy (citronellal-rich monoterpenoids in *C. citratus* and *O. suave*), moderate efficacy (limonoids in *A. indica*), and lower efficacy (1,8-cineole in *E. globulus*). Furthermore, the identification of minor compounds that correlate with sustained protection, such as those contributing to the unexpected field efficacy enhancement observed for *C. citratus*, could guide the development of optimized formulations with extended duration of action. This approach aligns with recent WHO recommendations for the development of evidence-based, locally sourced vector control interventions (WHO, 2023).

5.6 Implications for formulation development and future research

The integration of advanced chemometric and multivariate statistical approaches into the analysis of plant-based repellents has significant implications for the rational development of optimized formulations. By identifying the specific compounds and compound combinations that drive repellent efficacy, these methods enable the targeted selection of high-potential species and the design of synergistic blends that maximize protection while minimizing the required concentrations of active ingredients (Nguyen et al., 2025).

Recent studies have demonstrated that binary and ternary blends of botanical extracts can achieve significantly extended protection times compared to single extracts—up to 179 min of complete protection versus 67 min for individual extracts (Nguyen et al., 2025). The application of chemometric methods to identify synergistic compound combinations from the present dataset could similarly guide the development of optimized blends incorporating *C. citratus* and *O. suave*, which demonstrated high initial efficacy with *A. indica*, which showed superior sustained protection over 180 min (AUC 145). Such blends could potentially achieve both rapid onset and extended duration of protection, rivaling or exceeding the performance of synthetic repellents while maintaining the eco-friendly and culturally acceptable profile of plant-based products.

Furthermore, the unexpected finding of enhanced field efficacy for certain botanical extracts, particularly *C. citratus*, warrants further investigation using chemometric approaches that incorporate environmental variables (temperature, humidity, wind speed) as additional predictors in multivariate models. This would enable the

development of region-specific formulation recommendations tailored to the unique environmental conditions of Dire Dawa, Harari, and Somali regions.

5.7 Conclusion on chemical innovation

In conclusion, while the present study provides valuable baseline data on the chemical composition and repellent efficacy of four Ethiopian medicinal plants, the full potential of the chemical dataset remains unexplored. The incorporation of advanced chemometric and multivariate statistical approaches, including Principal Component Analysis, Hierarchical Cluster Analysis, Partial Least Squares regression, and comprehensive chemical fingerprinting, would substantially enhance the scientific impact and novelty of the work. These methods would enable the identification of key bioactive compounds and synergistic combinations, the prediction of efficacy from chemical profiles, and the rational design of optimized formulations for malaria prevention in resource-limited Ethiopian communities. Future studies should prioritize the application of these approaches to maximize the translational potential of Ethiopian medicinal plants for sustainable vector control.

6. Conclusions

This study provides robust scientific evidence for the mosquito-repellent potential of selected Ethiopian medicinal plants, with *Cymbopogon citratus* and *Ocimum suave* demonstrating protection levels comparable to the synthetic standard, DEET, during the initial 30–60 min of exposure. Phytochemical characterization identified citronellal as the principal bioactive constituent associated with repellent activity, and correlation analysis revealed a strong positive relationship between citronellal concentration and mosquito bite reduction, highlighting its importance as a key determinant of efficacy.

The experimental findings were supported by rigorous statistical analyses, which confirmed significant differences among treatments and demonstrated that plant species accounted for most of the observed variation in repellent performance. Furthermore, field evaluations conducted under natural environmental conditions in Dire Dawa, Harari, and the Somali Region validated the laboratory results, indicating that *C. citratus* remained highly effective for at least two hours and achieved substantial reductions in mosquito bites during peak vector activity. Although DEET provided longer-lasting protection, the botanical extracts exhibited comparable initial efficacy while offering important advantages, including local availability, affordability, environmental sustainability, and reduced dependence on synthetic chemical repellents.

Beyond validating traditional Ethiopian ethnobotanical knowledge, this work contributes to the growing body of evidence supporting plant-derived repellents as practical components of integrated vector management and malaria prevention programs. The enhanced efficacy observed under field conditions also suggests that environmental variables may influence the release and dispersion of volatile bioactive compounds, warranting further investigation.

Nevertheless, this study evaluated a limited number of plant species and focused primarily on short-term repellency. Future research should investigate formulation strategies capable of prolonging protection time, evaluate the stability of active compounds under diverse climatic conditions, assess safety through toxicological and dermatological studies, and conduct large-scale randomized field trials across different ecological settings and mosquito species. Such investigations will be essential for translating these promising botanical resources into standardized, evidence-based, and commercially viable mosquito repellent products suitable for widespread public health application.

Recommendations

Based on these findings, the following recommendations are proposed: First, integrate *Cymbopogon citratus* and *Ocimum suave* into community-based malaria control programs in the Dire Dawa, Harari, and Somali regions. Second, develop standardized formulations (e.g., topical creams, sprays, or smoke-generating preparations) to ensure consistent efficacy. Third, conduct long-term safety and stability studies to establish shelf-life and application guidelines. Fourth, establish local cultivation and processing cooperatives to ensure sustainable plant supply and community economic benefits. Fifth, conduct further research into synergistic combinations of these plants to extend the duration of protection. Finally, engage local health authorities and traditional practitioners to promote the adoption of these evidence-based botanical repellents alongside existing vector control measures.

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8. Authors' Contributions

Melaku Masresha Woldeamanuel: conceptualization, investigation, methodology, data curation, formal analysis, writing – original draft. *Belay Sitotaw Goshu*: investigation, methodology, data curation, validation, writing – review & editing. *Feven Tigistu Ayele*: investigation, data curation, visualization, writing – review & editing. *Shiemeles Ayalew Ali* (Supervisor): supervision, conceptualization, methodology, validation, resources, project administration, funding acquisition (if applicable), writing – review & editing.

9. Conflicts of Interest

The authors declare that they have no conflicts of interest regarding the publication of this manuscript.

10. Ethics Approval

Ethical approval for this study was granted by the Dire Dawa University Institutional Review Board (IRB) (Approval No. DDU-RTTO-2024-ETH). All study procedures were conducted in accordance with the approved ethical guidelines, and written informed consent was obtained from all participants before participation.

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Institutional Review Board Statement

This study was approved by the Dire Dawa University Institutional Review Board (IRB) (Approval No. DDU-RTTO-2024-ETH) and conducted in accordance with the principles of the Declaration of Helsinki and the approved ethical guidelines.

Informed Consent Statement

Written informed consent was obtained from all participants before enrollment in the study.

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