Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Larvicidal efficacy of selected medicinal plant extracts against larvae of medically important malaria and arbovirus vectors

Negesse Gebissa

negesse1973@gmail.com

Addis Ababa University

Ketema Tolossa

Addis Ababa University

Araya Gebresilassie

Addis Ababa University

Esayas Akililu

Addis Ababa University

Daniel Bisrat

Addis Ababa University

Barsisaa Kumsa

Addis Ababa University

Sisay Dugassa

Addis Ababa University

Article

Keywords:

Posted Date: November 10th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11271602/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Larvicidal efficacy of selected medicinal plant extracts against larvae of medically important malaria and arbovirus vectors

Negesse Gebissa[1*], Ketema Tolossa[1], Araya Gebresilassie[2], Esayas Aklilu[1], Daniel Bisrat[3], Bersissa Kumsa[4] and Sisay Dugassa[1*]

1 Aklilu Lemma Institute of Health Research, Addis Ababa University, Addis Ababa P.O. Box 1176, Ethiopia

2 Department of Zoological Sciences, College of Natural and Computational Sciences, Addis Ababa University, Addis Ababa P.O. Box 1176, Ethiopia

3 College of Health Sciences, Addis Ababa University, Addis Ababa P.O. Box 1176, Ethiopia

4 College of Veterinary Medicine and Agriculture, Addis Ababa University, Bishoftu P.O. Box 1176, Ethiopia

* Correspondence: negesse1973@gmail.com; sisaydlw2@gmail.com

ABSTRACT

Background & Objectives: Mosquitoes transmit serious human diseases, causing millions of deaths every year. The development of resistance to chemical insecticides has resulted in rebounding vectorial capacity, meaning that plants may serve as viable alternative sources of mosquito control agents. The present study critically tested the larvicidal activities of four candidate medicinal plant species: *Ocimum forskolei*, *Gymnema sylvestre*, *Lippia javanica,* and *Datura stramonium*. These species are traditionally used for mosquito control and are reported to be effective against larvae of mosquito vectors of malaria and arboviral diseases in Ethiopia. The extracts were evaluated against late third to early fourth instar larvae of *Anopheles arabiensis, Anopheles stephensi*, and *Aedes aegypti.*

Methods: Dried parts of the four selected medicinal plants were extracted using polar (80% methanol) and non-polar (n-hexane) solvents. The extracts were evaluated for larvicidal activities against late 3rd to early 4th instar larvae of *An. arabiensis*, *An. stephensi*, and *Ae. aegypti* mosquitoes. Larval mortalities were recorded after 24 hours of exposure following standard WHO bioassay guidelines.

Results: All n-hexane crude extracts showed varying degrees of larvicidal activity against *An. arabiensis.* The highest activities were observed in the n-hexane extracts of *G. sylvestre* and *L. javanica*, achieving 100% and 99.7% larval mortality at 2000 ppm, respectively. At 500 ppm, n-hexane crude extracts of *G. sylvestre* and *L. javanica* caused mortality rates ranging from 14.7% to 56.7%, whereas *O. forskolei* and *D. stramonium* at 1000 ppm caused mortalities ranging from 64.3% to 64.7%. N-hexane crude extracts of *G. sylvestre* and *L. javanica* at 2000 ppm concentration had similar effects to the standard larvicide temephos (100% mortality). Additionally, n-hexane crude extracts of *L. javanica* at 2000 ppm caused 100% *Ae. aegypti* and *G. sylvestre* 100% *An. arabiensis* larval mortality receptively. Probit analysis revealed that *G. sylvestre* and *L. javanica* were extremely potent against *An. arabiensis* larvae, with LC_{50} values of 921.00 ppm (LC_{90} = 1340.30 ppm) and 659.42 ppm (LC_{90} = 1034.47 ppm), respectively. *L. javanica* also exhibited strong potency against *Ae. aegypti* larvae with an LC_{50} of 1005.50 ppm and LC_{90} of 1503.90 ppm.

Conclusion: N-hexane crude extracts of the evaluated medicinal plants possess strong potential for use as eco-friendly botanical larvicides against *An. arabiensis* and *Ae. aegypti*. Further

studies to isolate, identify, and formulate the specific active compounds are highly recommended.

Keywords: Malaria, Arboviral diseases, Mosquitoes, *Anopheles arabiensis*, *Anopheles stephensi*, *Aedes aegypti,* Botanical Larvicides.

INTRODUCTION & BACKGROUND

Vector-borne diseases contribute significantly to the global burden of disease, accounting for approximately 20% of the tropical infectious disease burden and resulting in roughly 1 million human deaths annually [1]. The mortality rate is disproportionately higher in sub-Saharan Africa. Among disease vectors, mosquitoes represent a prominent threat to public health worldwide, transmitting deadly infections such as malaria, dengue fever, yellow fever, lymphatic filariasis, Zika virus, and chikungunya.

Interventions targeting breeding sites interrupt the disease transmission cycle by eliminating vector larvae before they emerge as flying adults [2]. Traditionally, vector control has relied heavily on synthetic chemical larvicides, including insect growth regulators (e.g., diflubenzuron, methoprene) and organophosphates (e.g., temephos, chlorpyrifos, fenthion).

Although synthetic chemical agents have successfully suppressed vector populations for decades, their long-term, extensive usage has triggered severe challenges. These include the widespread development of insecticide resistance among vector species, environmental degradation, and hazardous secondary effects on non-target organisms, including humans [2]. Consequently, global research has shifted toward plant extracts and phytochemicals as promising sources of biodegradable, eco-friendly, and effective mosquito larvicidal agents.

In Ethiopia, mosquito-borne diseases such as malaria, dengue fever and yellow fever remain major public health problems. *Anopheles arabiensis* is the primary malaria vector, while *Anopheles funestus*, *Anopheles pharoensis*, and *Anopheles nili* serve as secondary vectors [7]. Furthermore, *Anopheles stephensi*, an efficient urban malaria vector originally endemic to South Asia and the Middle East, was first reported in Ethiopia in 2016 and has since spread to several urban centers [8]. Ethiopia reports millions of confirmed malaria cases annually [9, 10], while dengue outbreaks have been documented in eastern and central parts of the country [10]. The continued expansion of mosquito vectors and the emergence of invasive species pose additional

challenges to vector control efforts [10, 11]. While conventional synthetic chemical insecticides—including deltamethrin, metofluthrin, acetamiprid, and cypermethrin—have historically provided effective vector control, [12] their prolonged and intensive use has triggered severe secondary crises. These include rapid resistance development in target populations, vector resurgence, persistent environmental contamination, and toxic bioaccumulation within non-target ecosystems [13]. Furthermore, exposure to these synthetic chemicals poses direct health risks to human populations, including endocrine disruption, reproductive toxicity, and potential carcinogenic effects [14, 15].

To mitigate these drawbacks, plant-derived phytochemicals have emerged as highly viable alternatives, with extensive global reviews highlighting their efficacy and general safety profiles in eradicating medically important mosquitoes [16, 17]. These botanical secondary metabolites are typically biodegradable, affordable, easily sourced, and possess broad-spectrum, target-specific modes of action that simultaneously disrupt the physiological and behavioral processes of vectors. Beyond crude extractions, advanced nanotechnology platforms have further utilized green-synthesized plant nanoparticles to achieve highly concentrated larvicidal thresholds against structural viral vectors [18], illustrating the diverse landscape of botanical vector-control technologies. Spurred by the urgent need for sustainable integrated vector management tools and building on global literature synthesizes on botanical agents [18]. Given the urgency of developing plant-derived alternatives, this study evaluated the larvicidal potential of polar (80% methanol) and non-polar (n-hexane) extracts of four traditionally used Ethiopian plant species: *Ocimum forskolei*, *Gymnema sylvestre*, *Lippia javanica*, and *Datura stramonium*. Testing was performed against the major vectors *An. arabiensis, An. stephensi,* and *Ae. aegypti* following standardized World Health Organization [3] bioassay protocols. The results establish foundational data for formulating sustainable botanical larvicides for large-scale mosquito control programs.

MATERIALS AND METHODS

Selection and Collection of Plant Species

An ethno-taxonomic approach was utilized to select promising larvicidal candidate plants by reviewing existing ethnobotanical literature, published articles, theses, and institutional research reports over the past two decades [4]. Scientific databases, including Google Scholar and

PubMed, were queried using keywords such as 'insecticide', 'larvicides', and 'medicinal plants'. Candidate plants were prioritized based on their traditional use reports, citation frequency (minimum of 3 distinct peer-reviewed articles), and calculated fidelity levels to evaluate their documented potential against insects [5]. Permission latter was obtained from Oromia regional State Biodiversity Office to collect the Selected plants.

Four plant species were selected: *O. forskolei*, *G. sylvestre, L. javanica* Hochst. ex Walp., and *D. stramonium* (Ait.) Benth. Field collection of voucher specimens and bulk plant materials was conducted during August and November 2024 across the Oromia Regional State (Jimma Zone, Asendabo District) and the Southwest Ethiopia Peoples' Regional State (Bonga District). All plant collections conformed strictly to established ethical guidelines for plant specimen procurement.

Voucher Specimen Collection and Identification

Taxonomic verification of all physical specimens was completed by botanist Mr. Melaku Wondafirash, with archival repositories established at the National Herbarium within Addis Ababa University's Department of Plant Biology and Biodiversity Management. The field data were recorded, summarized, and presented properly in Table 1.

Table 1: Eco-geographical and taxonomic records of the selected medicinal plant species (AO: Afan Oromo).

S.N	Species name	Voucher Numbers	Family name	Local name	Plant part	Habita t	GPS coordinates	Altitude
1	*O. forskolei*	LG-09	Lamiace ae	Hancabi (AO)	Aerial parts	Herb; garden	07°46'19.413"N (37°14'03.297"E)	1,754m
2	*G. sylvestre*	LG-10	Apocyna ceae	Dandaro (AO)	Aerial parts	Herb; field	07°06'42.24"N (36°29'11.97"E)	1,712m
3	*L. javanica*	LG-11	Verbena ceae	Koseret (AO)	Aerial parts	Herb; Home	07°46'13.715"N (037°14'04.492"E)	1,764m
4	*D. stramonium*	LG-12	Salicace ae	Farra goona (AO)	Seed	Shrub; Wild	07°46'14.359"N (037°14'02.239"E)	1,767m

Sample Collection and Processing

Specific plant structures—aerial parts for *O. forskolei, G. sylvestre,* and *L. javanica,* and leaves for *D. stramonium*—were collected separately in ventilated plastic containers and transported to the laboratory at the Aklilu Lemma Institute of Health Research (ALIHR), AAU. The materials

were shade-dried at room temperature (25±2°C) for 7 days. Once completely dried, the plant materials were pulverized into a uniform powder using an electrical grinder and passed through a 1-mm mesh sieve. This process maximized surface area and ensured homogenous solvent penetration during extraction. The final powders were preserved in labeled, airtight opaque bags until extraction.

Preparation of Crude Extracts

Measured masses of the finely powdered plant materials were subjected to cold maceration using 80% methanol and n-hexane solvents at a solute-to-solvent ratio of 1:5 (w/v) for 3 days. Continuous agitation at 120 rpm was maintained on an orbital shaker to optimize extraction kinetics. The mixture was filtered through a four-layered Miracloth to isolate the liquid micelle from the solid marc. Clarification was achieved by centrifuging the filtrate at 1,500 rpm for 5 minutes using a HERMLE (Z 383 K) centrifuge.

The organic solvent filtrates were concentrated using a vacuum rotary evaporator under reduced pressure at 40°C. Aqueous fractions were evaporated to absolute dryness inside a specialized incubator at 40°C. Traces of residual solvents were cleared by allowing the thick crude extracts to stand at ambient room temperature. The final dried masses were weighed to determine percentage extraction yields and stored in amber glass vials at 4°C for subsequent biological screening.

Larvicidal Bioassay

Larval bioassays on laboratory-reared mosquitoes were performed according to standard World Health Organization procedures [3]. Stock solutions (1% w/v) were formulated by dissolving 200 mg of each crude extract in 20 ml of 5% dimethyl sulfoxide (DMSO). Twofold serial dilutions were executed to obtain a range of operational concentrations: 2000, 1000, 500, 250, 125, and 62.5 ppm. The bioassays targeted the late 3rd to early 4th instar larvae of *Anopheles arabiensis, Anopheles stephensi,* and *Aedes aegypti.*

For each bioassay concentration, 100 larvae were evaluated across four replicates of 25 individuals each, alongside a parallel group of 25 larvae assigned to a positive control group (temephos larvicide). Each operational protocol was repeated across three independent trials. Larval mortality was recorded after a strict 24-hour exposure period [3]. An initial phase

screening was performed at 2000 ppm to categorize extract potency: strong (>80% mortality), moderate (60–80% mortality), weak (40–60% mortality), and little/no activity (<40% mortality). Extracts meeting or exceeding a 40% mortality threshold were advanced to comprehensive dose-response bioassays to compute LC_{50} and LC_{90} values [3].

Statistical Data Analysis

Dose-dependent mortality percentages were normalized, transformed, and subjected to One-Way Analysis of Variance (ANOVA) using SPSS Version 26. Post hoc mean comparisons were conducted via Tukey's honestly significant difference (HSD) test and Duncan's multiple range comparison. Data points are presented as mean percent mortality ± standard error of the mean (SEM). Statistical significance was recognized at $P < 0.05$. Lethal concentration values (LC_{50} and LC_{90}) alongside their corresponding 95% upper and lower confidence limits (CL) were calculated through a generalized linear probit model, incorporating Chi-square (X^2) verification.

RESULTS

Plant Species Extraction Yields

The extraction yields generated from the 80% methanol and n-hexane protocols varied across the four plant materials. The highest crude yield from 80% methanol maceration was obtained from *G. sylvestre* (11.3%), while the lowest was recorded from *O. forskolei* (4.5%). For the n-hexane extraction, *G. sylvestre* again produced the highest yield at 8.1%, whereas *D. stramonium* yielded the lowest at 2.8% (Table 2).

Table 2: Extraction yield percentages of selected medicinal plants utilizing polar and non-polar solvents.

S.N	Plant species	80% MeOH Yield (%)	n-hexane Yield (%)
1	*O. forskolei*	4.5%	7.3%
2	*G. sylvestre*	11.3%	8.1%
3	*L. javanica*	5.8%	6.0%
4	*D. stramonium*	9.7%	2.8%

Preliminary Larvicidal Screening

Preliminary screening at an exposure threshold of 2000 ppm demonstrated clear discrepancies between the solvents. All crude extracts derived from 80% methanol failed to meet the active threshold, with larval mortalities staying below 10%. In contrast, the n-hexane extracts displayed strong larvicidal action. At 2000 ppm, n-hexane extracts of *G. sylvestre* and *L. javanica* induced 100% and 99.7% mortality against *An. arabiensis,* respectively. *L. javanica* also caused 100% mortality against *Ae. aegypti. O. forskolei* and *D. stramonium* extracts produced moderate activity, both reaching 73.0% mortality against *An. arabiensis* (Table 3). No test extracts demonstrated meaningful larvicidal performance against *An. stephensi* during the initial screening.

Table 3: Preliminary larvicidal screening of plant extracts at 2000 ppm against late 3rd to early 4th instar larvae after 24 hours.

S.N	Plant extracts	Mosquito species	80% MeOH % Mortality ± SD	n-hexane % Mortality ± SD	5% DMSO % Mortality ± SD
1	*O. forskolei*	*An. arabiensis*	7 ± 1.00%	99.7 ± 1.20%	-100 ± 0.00%
		Ae. aegypti	3 ± 0.00%	100 ± 0.00%	-100 ± 0.00%
2	*G. sylvestre*	*An. arabiensis*	10 ± 1.20%	100 ± 0.00%	-100 ± 0.00%
3	*L. javanica*	*An. arabiensis*	3 ± 0.00%	73 ± 7.46%	-100 ± 0.00%
4	*D. stramonium*	*An. arabiensis*	3 ± 0.00%	73 ± 7.46%	-100 ± 0.00%

*****Footnote: Operational data labels reflect a standard 24-hour exposure duration. Baseline testing constants encompass n=4 replicates per individual trial over 3 independent trials. Negative control baseline boundaries consistently remained ≤ 2% mortality and were corrected via Abbott's formula.*

Dose-Response Evaluation

The active n-hexane extracts were subjected to structured dose-response evaluation across a range of lower concentrations. Larval mortality was strongly dose-dependent; effectiveness decreased markedly at lower treatment tiers, and zero mortality was recorded below 250 ppm. At a high concentration of 2000 ppm, *G. sylvestre* and *L. javanica* matched the 100% mortality efficiency of the positive standard check (0.25 ppm temephos). At a lower concentration of 500

ppm, *L. javanica* and *G. sylvestre* maintained clear insecticidal activity, with average larval mortalities of 14.7% for *G. sylvestre* against *An. arabiensis,* while *O. forskolei* and *D. stramonium* required 1000 ppm to cause noticeable mortality effects (Table 4).

Table 4: Mean percent mortality of active n-hexane extracts at varying concentrations after 24 h

Plant species	Mosquito species	Conc. (ppm)	Mean %	95% CI	
				Lower Boundary	Upper Boundary
		5% DMSO	-100.0	-100.00	-81.96
G. sylvestre	*An. arabiensis*	2000	100.0*	100.0	100.0
		1000	53.7*	41.4	96.0
		500	14.7*	10.5	92.0
		250	0.0*	0.0	4.0
L. javanica	*An. arabiensis*	2000	99.7*	98.9	100.0
		1000	77.7*	75.1	80.0
		500	56.7*	51.6	61.7
		250	0.0*	0.0	0.0
	Ae. aegypti	2000	100.0*	100.0	100.0
		1000	82.7*	80.1	85.6
		500	29.3*	24.1	34.6
		250	2.0*	-0.54	4.5
O. forskolei	*An. arabiensis*	2000	73.3*	68.3	77.8
		1000	64.3*	59.7	68.9
		500	0.0*	0.0	8.0
D. stramonium	*An. arabiensis*	2000	73.0*	68.2	77.8
		1000	64.7*	60.0	69.4
		500	0.0*	0.0	0.0
		Temephos 0.25	100*	100.00	81.96

Footnote: Operational data labels reflect a standard 24-hour exposure duration. Baseline testing constants encompass n=4 replicates per individual trial over 3 independent trials. Negative control baseline boundaries consistently remained ≤ 2% mortality and were corrected via Abbott's formula.

Lethal Concentration Determinations (LC_{50} and LC_{90})

Probit analysis confirmed that *L. javanica* and *G. sylvestre* possess the highest toxicity profiles. Against *An. arabiensis, L. javanica* was the most potent agent, yielding an LC_{50} value of 659.4 ppm and an LC_{90} value of 1034.5 ppm. *G. sylvestre* also demonstrated strong potency against *An. arabiensis* with an LC_{50} of 921.0 ppm and an LC_{90} of 1340.3 ppm. For *Ae. aegypti, L. javanica* displayed an LC_{50} of 1005.5 ppm and an LC_{90} of 1503.90 ppm. *O. forskolei* and *D. stramonium* displayed lower toxicity profiles, characterized by higher LC_{50} thresholds exceeding 1200 ppm (Table 5).

Table 5: Lethal concentrations (LC_{50} and LC_{90}) of active n-hexane extracts against mosquito larvae after 24 h.

Plant extracts	Mosquito species	X^2	LC_{50}	95% CI (LB/UB)	LC_{90}	95% CI (LB/UB)	P-value
G. sylvestre	*An. arabiensis*	2.4	921.0	800.5 / 1099.0	1340.3	1148.8 / 1708.0	0.003*
L. javanica	*An. arabiensis*	15.8	659.4	373.3 / 1394.0	1034.5	732.2 / 3100.8	0.380
	Ae. aegypti	5.4	1005.5	864.8 / 1216.7	1503.9	1276.7 / 1926.5	0.001*
O. forskolei	*An. arabiensis*	19.8	1267.4	671.8 / 3039.7	1972.9	1358.7 / 6769.4	0.542
D. stramonium	*An. arabiensis*	22.4	1329.9	646.5 / 4479.0	2098.4	1406.0/ 11293.9	0.772

Footnote: Operational data labels reflect a standard 24-hour exposure duration. Baseline testing constants encompass n=4 replicates per individual trial over 3 independent trials. Asterisks (*) denote statistical significance ($P < 0.05$), indicating a significant lack of fit for the Probit model.

DISCUSSION

The results demonstrate that non-polar botanical extracts possess potent insecticidal properties against major mosquito vectors in Ethiopia, offering a promising, eco-friendly alternative to synthetic chemical larvicides [5, 6]. Historically, vector control programs have relied heavily on

synthetic organophosphates like temephos to suppress larval populations [2]. However, the long-term, intensive deployment of these chemical agents has triggered widespread insecticide resistance among primary vectors, alongside severe ecological degradation and hazardous secondary impacts on non-target aquatic organisms [2, 7, 8]. Consequently, exploring the bioactive potential of local flora such as *G. sylvestre, L. javanica, O. forskolei*, and *D. stramonium* bridges a critical gap in the development of sustainable, biodegradable, and integrated vector management frameworks [9, 10].

Among the evaluated botanical species, the n-hexane extracts of *L. javanica* and *G. sylvestre* emerged as highly potent interventions against *An. arabiensis,* the predominant vector of malaria in Ethiopia [19]. At a concentration of 2000 ppm, both crude extracts achieved complete or near-complete larval knockdown within 24 hours of exposure, matching the 100% benchmark efficiency of the standard organophosphate larvicide, temephos. Probit modeling established *L. javanica* as the most potent agent against *An. arabiensis* with an LC_{50} value of 659.4 ppm and an LC_{90} value of 1034.5 ppm, closely followed by *G. sylvestre* (LC_{50} = 921.0 ppm; LC90 = 1340.3 ppm). The strong insecticidal activity of *L. javanica* can be attributed to its characteristic lipophilic secondary metabolites, such as volatile monoterpenes and sesquiterpenes, which have been documented to severely disrupt the insect nervous and respiratory systems [20, 21, 22]. Conversely, *O. forskolei* and *D. stramonium* exhibited lower toxicity profiles against *An. arabiensis,* characterized by significantly higher LC_{50} thresholds exceeding 1200 ppm. This indicates a lower density or reduced synergy of the necessary toxic active principles within these specific plant tissues.

Beyond malaria vector control, *L. javanica* demonstrated excellent cross-species efficacy by driving a 100% mortality rate against *Aedes aegypti* larvae at 2000 ppm. This multi-vector knockdown capability is highly significant given the rising public health burden of co-circulating arboviral diseases like dengue, yellow fever, and chikungunya across East Africa [23, 24, 25]. The calculated LC_{50} value for *L. javanica* against *Ae. aegypti* (1005.5 ppm) highlights its prospective utility as a broad-spectrum botanical larvicide capable of suppressing diverse aquatic vector populations simultaneously [26].

Intriguingly, none of the tested extracts demonstrated notable larvicidal performance against *An. stephensi* within the evaluated parameters, failing to meet the active screening threshold during

initial trials. This indicates a profound variation in species-specific physiological tolerance and detoxification mechanisms [27]. *An. stephensi* is a highly invasive, urban-adapted malaria vector rapidly expanding its footprint across the Horn of Africa and Yemen [27]. Its observed resilience to these extracts may be linked to elevated levels of metabolic enzymes, such as cytochrome P450 monooxygenases or esterases, which are known to neutralize botanical toxins [7]. This species-specific insensitivity highlights the necessity for deeper biochemical investigation to map out vector-toxin interactions.

CONCLUSION AND RECOMMENDATIONS

This study confirms that n-hexane crude extracts of *G. sylvestre, L. javanica, O. forskolei*, and *D. stramonium* hold clear promise as prospective botanical larvicides against *An. arabiensis* larvae. Furthermore, the leaf and aerial extracts of *L. javanica* represent a potent candidate tool against *Ae. aegypti*. These local botanical options offer a route to develop effective, biodegradable, and cost-effective alternatives for vector control, supporting insecticide resistance management frameworks in Ethiopia.

Based on these findings, the following actions are recommended for future research and development:

- Conduct advanced chromatographic and spectroscopic analyses (such as GC-MS or HPLC) to isolate and identify the specific non-polar bioactive fractions responsible for larval toxicity.
- Develop standardized emulsifiable concentrate (EC) or granular formulations to enhance water solubility, stability, and field delivery in lentic breeding habitats.
- Evaluate the field efficacy and residual toxicity profiles of these formulations across natural, ecologically diverse breeding sites.
- Perform comprehensive ecotoxicological bioassays to confirm safety profiles regarding non-target aquatic organisms, beneficial insects, and human populations.

Metadata

Author Contributions: Conceptualization, N.G. and S.D.; methodology, N.G., K.T. and A.G.; data curation, E.A. and D.B.; writing—original draft preparation, N.G.; writing—review and

editing, S.D. and B.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Office of the Vice President for Research and Technology Transfer, Addis Ababa University.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Declaration of Conflicting Interests

The authors declare that they have no competing interests or conflicts of interest regarding the research, authorship, and publication of this article.

Ethics Approval

Field data collection was conducted following approval by the Institutional Research Ethics Review Committee (ALIPB-IRERC) of the Aklilu Lemma Institute of Pathobiology, Addis Ababa University (Reference No. ALIP-IRERC/140/2024).

REFERENCES

1. World Health Organization. Global burden of vector-borne diseases. Geneva: World Health Organization; 2017.
2. A, et al. Synthetic and plant-derived larvicides in vector management. J Econ Entomol. 2017; 110(3):124-132. World Health Organization. Guidelines for laboratory and field testing of mosquito larvicides. Geneva: World Health Organization; 2005. Report No.: WHO/CDS/WHOPES/GCDPP/2005.13.
3. Matiin A. Reviewing ethnobotanical selection methodologies for plant-derived larvicides over two decades. Ethnobot Res. 1995; 4(2):89-101.
4. Friedman J, Yaniv Z, Dafni A, Palewitch D. A preliminary classification of the healing potential of medicinal plants, based on a rational analysis of an ethno-

pharmacological field survey among Bedouins in the Negev Desert, Israel. J Ethnopharmacol. 1986; 16(2-3):275-287.

5. Shaalan EA, Canyon D, Younes MWF, Abdel-Wahab H, Mansour A. A review of botanical phytochemicals with mosquitocidal potential. Environ Int. 2005; 31(8):1149-1166.
6. Zoubiri S, Baaliouamer A. Potentiality of plants as source of insecticide principles. J Saudi Chem Soc. 2014; 18(6):925-929.
7. Senthil-Nathan S. A review of resistance mechanisms of synthetic insecticides and botanicals phytochemicals, and essential oils as alternative larvicidal agents against mosquitoes. Front Physiol. 2020; 10:1591.
8. Nicolopoulou-Stamati P, Maipas S, Kotampasi C, et al. Chemical pesticides and human health: the urgent need for a new concept in agriculture. Front Public Health. 2016; 4:148.
9. Kamaraj C, Rahuman AA, Mahapatra A, Bagavan A, Elango G. Insecticidal and larvicidal activities of medicinal plant extracts against mosquitoes. Parasitol Res. 2010; 107(6):1337-1349.
10. Engdahl CS, Tikhe CV, Dimopoulos G. Discovery of novel natural products for mosquito control. Parasit Vectors. 2022; 15(1):481.
11. Devine GJ, Vazquez-Prokopec GM, Bibiano-Marín W, Pavia-Ruz N, Che-Mendoza A, Medina-Barreiro A, et al. The entomological impact of passive metofluthrin emanators against indoor Aedes aegypti: A randomized field trial. *PLoS Negl Trop Dis*. 2021; 15:e0009036. https://doi.org

12. Singhi M, Joshi V, Sharma R, Sharma K. Ovipositioning behaviour of Aedes aegypti in different concentrations of latex of Calotropis procera: studies on refractory behaviour and its sustenance across gonotrophic cycles. Dengue Bulletin. 2004; 28:184–188. Access URL: https://apps.who.int/iris/handle/10665/163695
13. Uwaifo F, John-Ohimai F. Dangers of organophosphate pesticide exposure to human health. *Maternal and Child Science Medicine*. 2020; 4:27. DOI: 10.4103/MTSM.MTSM_2_20
14. Pratiwi MAM, Purwati. The repellent activity test of rosemary leaf (Rosmarinus officinalis L.) essential oil gel preparations influence on Aedes aegypti mosquito. Journal of Physics: Conference Series. 2021; 1788:012016. DOI: 10.1088/1742-6596/1788/1/012016
15. Govindarajan M, Rajeswary M, Hoti SL, Benelli G. Larvicidal potential of carvacrol and terpinen-4-ol from the essential oil of Origanum vulgare (Lamiaceae) against Anopheles stephensi, Anopheles subpictus, Culex quinquefasciatus and Culex tritaeniorhynchus (Diptera: Culicidae). *Research in Veterinary Science*. 2016; 104:77–82. DOI: 10.1016/j.rvsc.2016.01.011
16. Dass K. Evaluation and efficacy of plant extracts in eradicating medically important mosquitoes: a review. *Toxicology and Environmental Health Sciences*. 2024; 16(4):413-458. Doi: 10.1007/s13530-024-00214-y.
17. Dass K, Prakash N, Manogar P, Murugesan R. Current insights and future perspectives of in silico molecular docking in dengue virus proteins inhibition: A

review. *Aspects of Molecular Medicine*. 2024; 4:100050. doi:10.1016/j.amolm.2024.100050.

18. Dass K, Murugesan R, Prabhu S. Phyto compounds as Medically Important Mosquito Vector Control Agents: A Review. *Springer Nature*.2026. DOI: 10.1007/s40011-026-01797-
19. Tiwary M, Naik SN, Tewary DK, et al. Chemical composition and larvicidal activities of the essential oil of *Zanthoxylum armatum* DC (Rutaceae) against three mosquito vectors. J Vector Borne Dis. 2007; 44(3):198-204.
20. Bossou AD, Mangelinckx S, Yedomonhan H, et al. Chemical composition and insecticidal activity of plant essential oils from Benin against *Anopheles gambiae* (Giles). Parasit Vectors. 2013; 6(1):337.
21. Caraballo H, King K. Emergency department management of mosquito-borne illness: malaria, dengue, and West Nile virus. Emerg Med Pract. 2014; 16(5):1-23.
22. Gedefie A, Debash H, Kassaw AB, et al. Epidemiology of arboviruses in humans and livestock in Ethiopia: a systematic review and meta-analysis. BMC Infect Dis. 2025; 25(1):458.
23. Lim A, Shearer FM, Sewalk K, et al. The overlapping global distribution of dengue, chikungunya, Zika and yellow fever. Nat Commun. 2025; 16(1):3418.
24. Choochote W, Chaithong U, Kamsuk K, et al. Adulticidal activity against *Stegomyia aegypti* (Diptera: Culicidae) of three *Piper* spp. Rev Inst Med Trop S Paulo. 2006; 48(1):33-37.

25. Merdya M, Sisay D, et al. Insecticidal effects of some selected plant extracts against *Anopheles stephensi* (Culicidae: Diptera). Malar J. 2022; 21(1):295.
26. Ebhodaghe FI, Sanchez-Vargas I, Isaac C, Foy BD, Hemming-Schroeder E. Sibling species of the major malaria vector *Anopheles gambiae* display divergent preferences for aquatic breeding sites in southern Nigeria. Malar J. 2024; 23(1):60.
27. Al-Eryani SM, Irish SR, Carter TE, et al. Public health impact of the spread of *Anopheles stephensi* in the WHO Eastern Mediterranean Region countries in Horn of Africa and Yemen: need for integrated vector surveillance and control. Malar J. 2023; 22(1):187.