

Bioinsecticidal Properties of extracts from Eight Medicinal Plants against Key malaria and viral mosquito vectors

Negesse Gebissa

negesse1973@gmail.com

Addis Ababa University

Ketema Tolossa

Addis Ababa University

Araya Gebresilassie

Addis Ababa University

Esayas Aklilu

Addis Ababa University

Daniel Bisrat

Addis Ababa University

Bersissa Kumsa

Addis Ababa University

Sisay Dugassa

Addis Ababa University

Research Article

Keywords: Arboviral diseases, Bioinsecticidal activities, Malaria, Medicinal plants, Mosquitoes

Posted Date: September 25th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11005515/v1>

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

[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Background: Escalating environmental concerns and widespread resistance to synthetic insecticides have intensified the search for sustainable, plant-based alternatives. Botanical bioinsecticides offer a safer profile for non-target organisms and leave minimal environmental residue while effectively suppressing target vector populations.

Methods Crude extracts from eight native medicinal plants—*Ocimum lamiifolium*, *Lepidium sativum*, *Amaranthus hybridus*, *Premna schimperi*, *Ocimum gratissimum*, *Argemone mexicana*, *Lippia adoensis*, and *Calpurnia aurea*—were prepared using 80% methanol & n-hexane solvents. Larvicidal and adulticidal bioassays were conducted under controlled laboratory conditions at concentrations ranging from 62.5200–2000 ppm against *An. arabiensis*, *An. stephensi*, and *Ae. aegypti*. Mortality data were recorded after 24 hours and evaluated via probit analysis to determine LC_{50} & LC_{90} values.

Results Among the tested botanicals, *O. gratissimum* and *A. mexicana* exhibited the highest larvicidal potency against *An. arabiensis*, yielding LC_{50} & LC_{90} ranges of 596.51–1084.48 ppm and 650.88–1039.50 ppm, respectively. These were followed by *L. adoensis* (LC_{50}/LC_{90} : 763.03–1158.01 ppm) and *A. mexicana* (LC_{50}/LC_{90} : 768.00–995.17 ppm) against *Ae. aegypti*. Notably, *O. gratissimum*, *A. mexicana*, and *C. aurea* induced 100% larval mortality in *An. arabiensis* at peak concentrations. Furthermore, n-hexane crude extracts of *O. lamiifolium*, *L. sativum*, *A. hybridus*, *P. schimperi*, *A. mexicana*, *L. adoensis*, and *C. aurea* demonstrated robust adulticidal efficacy against both *An. arabiensis* and *Ae. aegypti* across the evaluated concentration spectrum.

Conclusions the n-hexane and methanolic crude extracts of these evaluated plants demonstrate strong potential for development as eco-friendly bioinsecticides against major malaria and arboviral vectors. Future research should focus on isolating and characterizing the specific active phytochemical constituents driving this bioactivity.

INTRODUCTION

Mosquito-borne diseases represent a monumental public health challenge in tropical and subtropical regions worldwide, causing clinical manifestations that range from asymptomatic infections to severe, fatal outcomes [1]. For instance, chikungunya virus infections frequently result in debilitating, chronic arthralgia that persists for years, while Zika virus infections during pregnancy are directly linked to severe congenital neurological abnormalities [2]. Malaria continues to severely impede the socioeconomic development of endemic communities by crippling workforce productivity and imposing unsustainable healthcare costs, with young children bearing the heaviest burden of morbidity and mortality. Concurrently, lymphatic filariasis affects over 120 million people globally, causing permanent, disfiguring disabilities such as elephantiasis [3]. Collectively, vector-borne pathologies claim over one million lives annually [4]. Given the profound global burden of these diseases, establishing highly effective vector suppression strategies and personal protection measures is paramount.

While conventional synthetic chemical insecticides—including deltamethrin, metofluthrin, acetamiprid, and cypermethrin—have historically provided effective vector control, [5, 6, 7] 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 [8]. Furthermore, exposure to these synthetic chemicals poses direct health risks to human populations, including endocrine disruption, reproductive toxicity, and potential carcinogenic effects [9, 10].

To mitigate these drawbacks, plant-derived phytochemicals have emerged as highly viable alternatives. 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 [11]. Spurred by the urgent need for sustainable integrated vector management tools, this study evaluated the larvicidal and adulticidal potential of crude extracts obtained from eight culturally and medicinally significant Ethiopian plants: *O. lamiifolium*, *L. sativum*, *A. hybridus*, *P. schimperi*, *O. gratissimum*, *A. mexicana*, *L. adoensis*, and *C. aurea*. The bioinsecticidal efficacy of these extracts was systematically screened against three medically important mosquito vectors: *An. arabiensis*, *An. stephensi*, and *Ae. aegypti*.

Materials and Methods

Plant Selection, Collection, Authentication, and Processing

An ethno-taxonomic strategy was implemented to choose the targeted flora based on an extensive search of botanical families recorded in past literature. The select baseline data for these mosquito-control and therapeutic plants was drawn directly from peer-reviewed publications. To refine our selection criteria, we prioritized candidate species based on their citation prevalence—requiring documentation in no fewer than three independent papers—alongside the availability of robust contextual data. Guided by this initial literary matrix, field sampling expeditions were organized. A total of eight target plants (*O. lamiifolium*, *L. sativum*, *A. hybridus*, *P. schimperi*, *O. gratissimum*, *A. mexicana*, *L. adoensis*, and *C. aurea*) were gathered from home stead and wild populations based on documented domestic insecticidal uses. Both research extraction materials and authentic voucher specimens were secured during field tracking across August and November 2024. Collection coordinates spanned the Asendabo district (Jimma zone, Oromia Regional State) and the Bonga district (Southwest Ethiopia Regional State). 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
Plant material used in the study, including local name Afan Oromo (AO) names.

Plant name	Family	Plant part	Local name (AO)	Collection coordinates	Elevation (m)	Ref.
<i>O. lamiifolium</i> Hochst. Ex. Walp (LG-01)	Lamiaceae	Aerial parts	Damakase	(07°46'15.155"N) (037°14'03.58"E)	1,761	12, 13, 14
<i>L. sativum</i> L. (LG-10)	Brassicaceae	Seed	Feto	(07°43'20.34"N) (36°18'09.79"E)	1,772	15, 16, 17
<i>A. hybridus</i> L. (LG-06)	Amaranthaceae	Aerial parts	Asangira	(07°43'20.34"N) (36°18'09.79"E)	1,772	18, 19, 20
<i>P. schimperi</i> Engl. (LG-04)	Lamiaceae	Leaves	Urgessa	(07°46'05.85"N) (37°14'02.22"E)	1,757	21, 22, 23
<i>O. gratissimum</i> L (LG-02)	Lamiaceae	Aerial parts	Hancabi	(07°46'19.41"N) (37°14'03.29"E)	1,754	24, 25, 26
<i>A. mexicana</i> (LG-09)	Papaveraceae	Aerial parts	Dandaro	(07°46'22.24"N) (37°29'11.97"E)	1,712	27, 28, 29
<i>L. adoensis</i> Hochst. (LG-03)	Verbenaceae	Aerial parts	Koseret	(07°46'13.715"N) (37°14'04.49")	1,764	30, 31, 32
<i>C. aurea</i> (Ait.) (LG-05)	Fabaceae	Leaves	Cheka	(07°46'14.359"N) (37°14'02.24"E)	1,767	33, 34, 35

The distinct plant organs collected were segregated into separate breathable plastic containers, secured tightly, and transferred safely to the laboratories at the Aklilu Lemma Institute of Health Research (AAU). Preservation involved a 7-day ambient desiccation protocol under shade at an institutional temperature of $25 \pm 2^\circ\text{C}$. Following thorough drying, the biomass was pulverized using a mechanical laboratory mill. The resulting matrix was pushed through a 1-mm calibration sieve to generate a standardized particle distribution. This mechanical homogenization was designed to optimize sample uniformity, maximize the total exposed surface area, and break down cellular barriers to maximize extraction solvent permeation. The sealed, labeled powders were kept in light-insulated dry containers at ambient room temperature until extraction. Preparation of Extracts For the initial extraction, separate 100 g samples of the dry, sieved plant powders were placed in individual flasks containing either 80% methanol or pure n-hexane at an exact 1:5 (w/v) sample-to-solvent ratio. Agitation was maintained via a continuous orbital shaker operating at 120 rpm for 72 hours under room temperature conditions to ensure complete target metabolite leaching. The resulting liquid mixture (micelle) was isolated from the solid spent residue (marc) via vacuum filtration through four layers of dense miracloth. To clarify the liquid phase further, the

filtration yield was ran through a HERMLE centrifugal system (Model Z 383 K) running at 1500 rpm for 5 minutes. The supernatant phase was subsequently recovered and concentrated using a vacuum rotary evaporator system operating at a temperature limit of 40°C. Any remaining heavy aqueous fractions were transferred to labeled amber glass jars and brought to total dryness using a laboratory oven calibrated to 40°C. The net mass of the dry residues was recorded to compute extraction yields, and stock concentrations were preserved in amber vials at 4°C. From the primary 1% stock matrices, a broad serial concentration gradient spanning 62.5–2000 ppm was calculated and prepared to test both larval and mature target mosquito cohorts.

Colony Establishment and Mosquito Rearing

The experimental mosquito cohorts were sourced directly from the specialized insectary facilities maintained at Addis Ababa University's Aklilu Lemma Institute of Health Research. To initiate the rearing cycles, viable eggs of *An. arabiensis*, *Ae. aegypti*, and *An. stephensi* were submerged in shallow development trays containing dechlorinated tap water under monitored laboratory conditions. *Stephensi* were submerged in shallow development trays filled with dechlorinated tap water under monitored laboratory conditions. Eclosion into first-instar larval populations occurred consistently within a 24-hour incubation window. To support larval development and vitality, a standardized nutritional blend comprising pulverized sterile yeast and commercial dog biscuits blended at an exact 1:1 ratio was introduced daily. The bioassays were strictly restricted to late third-to-early fourth instar larvae, alongside young mature adults aged between 2 and 5 days post-emergence. To ensure data reproducibility and eliminate environmental variables, conditions within the separate insectary rooms were held constant at an ambient temperature of $25 \pm 2^\circ\text{C}$, relative humidity thresholds of $60\% \pm 10\%$, and an automated 12:12-hour light-dark photoperiod regime.

Experimental Bioassay Protocols

The biological screenings evaluated the larvicidal potential of four specific botanical candidates (*O. gratissimum*, *A. mexicana*, *L. adoensis*, and *C. aurea*). Concurrently, the adulticidal efficacy of all seven plant profiles (*O. lamiifolium*, *L. sativum*, *A. hybridus*, *P. schimperi*, *A. mexicana*, *L. adoensis*, and *C. aurea*) was benchmarked against mature vectors. Both evaluation pathways relied on established baseline guidelines set by the World Health Organization (WHO) protocols [36]. To set up the concentration gradients, 20 mL of a primary 1% (w/v) master stock solution was prepared for each plant extract. The uniform dissolution of the fractions was achieved via continuous low-speed mechanical stirring under room temperature conditions. Lower target exposure steps were systematically achieved by applying serial dilution mechanics dictated by the definitive concentration balance formula $C_1V_1 = C_2V_2$ [36].

Every distinct concentration-response trial required a baseline pool of 150 test specimens. For the active treatment arms, 100 individual organisms were divided uniformly across four matching replicates (containing 25 larvae or adults per cohort). The remaining 50 organisms were split equally to serve as

baseline reference groups, with 25 specimens assigned to the negative control bracket and 25 specimens evaluated under the positive control framework. The entire experimental matrix was executed in three independent chronological blocks. Following a definitive 24-hour exposure period, clear mortality tallies were logged across all test containers to calculate final percentage mortality rates using the standard equation [37].

$$\text{Mortality (\%)} = (\text{No. of larva/adult dead} / \text{No. of larvae/adult}) * 100$$

The efficacy of plant extracts was classified according to mortality rates as follows: strong activity (> 80% mortality), moderate activity (60–80%), weak activity (40–60%), and inactive or low activity (< 40%).

For larvicidal assays, temephos (0.25%) was used as the positive control, while 5% dimethyl sulfoxide (DMSO) served as the negative control. For adulticidal assays, acetone was used as the negative control. A synthetic insecticide was not included as a positive control for adult mosquitoes because resistance to commonly used adult mosquito insecticides has been widely reported [38].

During the initial screening tier, both larval and adult cohorts were exposed to a ceiling concentration of 2000 ppm to map baseline plant toxicity profiles. Efficacy thresholds were strictly grouped into four operational categories based on recorded kill rates: strong action (mortality > 80%), moderate performance (mortality between 60% and 80%), weak performance (mortality between 40% and 60%), and negligible state (mortality < 40%). Fractions that generated mortality rates exceeding the 40% boundary were immediately advanced to full scale, multi-step dose-response trials.

Statistical Evaluation

Experimental data are presented as mean percentage mortality values complemented by standard deviations. To identify statistically significant variances in average larvicidal and adulticidal mortality rates among the different crude extract treatments, a one-way analysis of variance (ANOVA) was executed using the SPSS software suite (Windows version 26). Inter-group variations were considered statistically significant if the calculated P-value fell below 0.05 ($P < 0.05$) or if there was no overlap between the 95% confidence intervals. Pairwise comparisons tracking mortality metrics among active extract doses, negative controls, and positive references were determined via Tukey's Honest Significant Difference (HSD) post-hoc test. Cumulative mortality determinations across twelve independent replicates were integrated to calculate the final 24-h post-exposure death rates. To establish the definitive median lethal concentration LC_{50} and 90% lethal concentration LC_{90} thresholds along with their associated upper and lower 95% confidence limits, the average insect mortality figures were subjected to a generalized linear probit regression model.

RESULTS

Phytochemical profiles and extract yields

The selection of eight medicinal plant species belong to six families, with Lamiaceae representing 37.5% (n = 3) and the rest divided equally among Brassicaceae, Amaranthaceae, Papaveraceae, Verbenaceae, and Fabaceae (12.5% each). Crude extraction yields varied by solvent polarity. Polar 80% methanol yields ranged from 4.50% (*O. gratissimum*) to 13.30% (*P. schimperi*). Non-polar n-hexane yields ranged from 2.80% (*C. aurea*) to 13.40% (*A. hybridus*) (Table 2).

Table 2
Percentage yield of 80% methanol and n-hexane crude extracts of targeted plant species evaluated against target larvae and adults mosquito vectors.

S.N	Plant species	Percentage Yields of extracts (% w/w)	
		80% Methanol	n-hexane
1	<i>Ocimum lamiifolium</i>	5.31	4.70
2	<i>Lepidium sativum</i>	11.30	8.10
3	<i>Amaranthus hybridus</i>	9.70	13.40
4	<i>Premna schimperi</i>	13.30	4.80
5	<i>Ocimum gratissimum</i>	4.50	7.30
6	<i>Argemone mexicana</i>	12.10	8.00
7	<i>Lippia adoensis</i>	5.80	6.00
8	<i>Calpurnia aurea</i>	9.70	2.80

Larvicidal Activity of Plant Extracts

The larvicidal activity of crude extracts from eight medicinal plant species was evaluated against third- to early fourth-instar larvae of *An. arabiensis*, *An. stephensi*, and *Ae. aegypti*. Preliminary screening identified the n-hexane crude extracts of *O. gratissimum*, *A. mexicana*, *L. adoensis*, and *C. aurea* as the most promising candidates for detailed larvicidal evaluation.

These active extracts were subsequently assessed in concentration–response bioassays at concentrations ranging from 62.5 to 2000 ppm. Larval mortality was recorded after 24 h of exposure, and the results are summarized in Table 3. All four n-hexane extracts exhibited concentration-dependent larvicidal activity against the three mosquito species, with mortality increasing significantly as extract concentration increased. The magnitude of activity differed among plant species and mosquito vectors, indicating species-specific susceptibility to the tested extracts. In contrast, the remaining four medicinal plants (*O. lamiifolium*, *L. sativum*, *A. hybridus*, and *P. schimperi*) had been evaluated previously and are therefore not presented here [39].

Table 3

Mean ($\pm$ SD) % mortality of late 3rd – early 4th instar the mosquito larvae exposed to different concentrations of n-hexane crude plant extracts after 24 h.

Plant Species	Mosquito species	Conc. (PPM)	Mean Mortality (%) $\pm$ SD	MD	95% CI		Sig.
					LB	UP	
<i>O. gratissimum</i>	<i>An. arabiensis</i>	5% DMSO	0.0 $\pm$ 0.00	1.00	118.04	81.96	1.00
		2000	100.0 $\pm$ 0.00 *	-100.0*	-118.04	-81.96	.000
		1000	86.90 $\pm$ 3.12*	-86.90*	-108.7	-72.63	.000
		500	26.93 $\pm$ 2.45 *	-26.93*	-59.3	-23.29	.000
		250	17.70 $\pm$ 1.88 *	17.7*	-44.7	-8.63	.005
		125	10.10 $\pm$ 1.10*	-10.1	-31.7	4.37	.547
		62.5	1.66 $\pm$ 0.33*	-1.66	-19.7	16.37	1.00
	<i>Ae. aegypti</i>	2000	87.50 $\pm$ 2.95 *	-87.5*	-113.54	-79.96	.000
		1000	73.70 $\pm$ 4.10*	-73.7*	-103.29	-69.71	.000
		500	5.30 $\pm$ 0.85*	-5.3*	-1.79	-8.21	.000
		250	0.0 $\pm$ 0.00	-13.5	-30.29	3.29	.999
		125	0.0 $\pm$ 0.00	-3.5	-20.29	13.29	1.00
		62.5	0.0 $\pm$ 0.00	.00	-16.79	16.79	1.00
<i>A. mexicana</i>	<i>An. arabiensis</i>	2000	100.0 $\pm$ 0.00*	-100.0*	-106.17	-77.16	.000
		1000	88.97 $\pm$ 2.10*	-88.97*	-99.17	-70.16	.000
		500	26.2 $\pm$ 1.95*	-26.2*	-30.17	5.84	.585
		250	11.3 $\pm$ 1.15*	-11.3*	-16.51	12.51	1.00
		125	5.0 $\pm$ 0.80*	-5.00	-12.17	16.84	1.00

Note: Values represent the mean of triplicate bioassays; (*) indicates data points with statically significant differences compared to the control group.

		62.5	$2.3 \pm 0.45^*$	-2.3	-106.17	-77.16	1.00
	<i>An. stephensi</i>	2000	$99.50 \pm 0.50^*$	-99.7*	-101.81	-97.52	.000
		1000	$16.20 \pm 1.35^*$	-16.0*	-19.15	-4.85	.000
		500	$.70 \pm 0.15^*$	-0.7	-2.81	1.48	.970
		250	$.40 \pm 0.05^*$	– .40	-2.48	1.81	1.00
		125	0.0 ± 0.00	.00	-2.15	2.15	1.00
		62.5	0.0 ± 0.00	.00	-2.15	2.15	1.00
	<i>Ae. aegypti</i>	2000	$100.0 \pm 0.00^*$	-100.0*	-102.31	-97.69	.000
		1000	$90.30 \pm 1.95^*$	-90.3*	-93.31	-88.69	.000
		500	$7.00 \pm 0.90^*$	-7.00*	-10.31	-5.69	.000
		250	0.0 ± 0.00	.00	-2.31	2.31	1.00
		125	0.0 ± 0.00	.00	-2.31	2.31	1.00
		62.5	0.0 ± 0.00	.00	-2.31	2.31	1.00
<i>L. adoensis</i>	<i>An. arabiensis</i>	2000	$79.60 \pm 3.40^*$	-79.6*	-85.36	-70.30	.000
		1000	$53.20 \pm 0.00^*$	-53.2	-67.03	50.03	.00
		500	$16.40 \pm 0.00^*$	-16.7	-4.70	3.36	1.00
		250	0.0 ± 0.00	.00	-4.03	4.03	1.00
		125	0.0 ± 0.00	.00	-4.03	4.03	1.00
		62.5	0.0 ± 0.00	.00	-4.03	4.03	1.00
	<i>An. stephensi</i>	2000	$100.0 \pm 0.00^*$	-100.0*	-109.11	-90.89	.000
		1000	$71.4 \pm 1.15^*$	-71.4*	-81.44	-53.22	.000
		500	$8.0 \pm 0.45^*$	-8.0	-14.11	4.11	.685
		250	$.4 \pm 0.05^*$	– .4	-9.44	8.78	1.00
		125	0.0 ± 0.00	.00	-9.11	9.11	1.00

Note: Values represent the mean of triplicate bioassays; (*) indicates data points with statically significant differences compared to the control group.

		62.5	0.0 ± 0.00	.00	-9.11	9.11	1.00
	<i>Ae. aegypti</i>	2000	100.0 ± 0.00*	-100.0*	-104.50	-95.50	.000
		1000	72.8 ± 2.10*	-72.8*	-87.83	-70.83	.000
		500	32.0 ± 1.65*	-32.3*	-43.83	-24.83	.000
		250	2.5 ± 0.20*	-2.5	-6.50	2.50	.864
		125	0.0 ± 0.00	.00	-4.50	4.50	1.00
		62.5	0.0 ± 0.00	.00	-4.50	4.50	1.00
<i>C. aurea</i>	<i>An. arabiensis</i>	2000	100.0 ± 0.00*	-100.0*	-106.21	-93.79	.000
		1000	73.7 ± 2.85*	-73.7*	-87.21	-59.79	.000
		500	15.7 ± 1.10*	-15.7*	-18.54	-6.13	.067
		250	0.0 ± 0.00	.00	-6.21	6.21	1.00
		125	0.0 ± 0.00	.00	-6.21	6.21	1.00
		62.5	0.0 ± 0.00	.00	-6.21	6.21	1.00
	<i>Ae. aegypti</i>	2000	71.4 ± 1.95*	-71.4*	-74.81	-68.86	.000
		1000	60.0 ± 1.40*	-60.3*	-62.81	-57.86	.000
		500	1.3 ± 0.15*	-1.3	-5.81	3.14	.988
		250	0.0 ± 0.00	.00	-4.47	4.47	1.00
		125	0.0 ± 0.00	.00	-4.47	4.47	1.00
		62.5	0.0 ± 0.00	.00	-4.47	4.47	1.00
		Temephos 0.25	100 ± 0.00*	-100.0	-118.04	-81.96	.000
Note: Values represent the mean of triplicate bioassays; (*) indicates data points with statically significant differences compared to the control group.							

The mean larval mortality differed significantly among concentrations of the n-hexane crude plant extracts compared with the negative control ($P < 0.05$), with significant effects becoming evident at concentrations ≥ 500 ppm (Table 3). All evaluated extracts exhibited concentration-dependent larvicidal activity, with mortality increasing progressively as extract concentration increased. At the highest concentration tested (2000 ppm), the n-hexane crude extracts produced complete (100%) mortality of *An. arabiensis* larvae after 24 h of exposure ($P < 0.001$).

Among the evaluated extracts, *O. gratissimum* and *A. mexicana* were the most potent against *An. arabiensis*, with LC₅₀ values ranging from 596.51–1084.48 ppm and LC₉₀ values from 650.88–1039.50 ppm. Similarly, *L. adoensis* exhibited pronounced larvicidal activity against *Ae. aegypti*, with LC₅₀ and LC₉₀ values ranging from 763.03–1158.01 ppm and from 768.00–995.17 ppm, respectively.

At 2000 ppm, *O. gratissimum*, *A. mexicana*, and *C. aurea* caused complete (100%) mortality of *An. arabiensis* larvae, whereas *A. mexicana* and *L. adoensis* produced nearly complete mortality against *An. stephensi* and *Ae. aegypti*. No mortality was recorded in the negative control, while the positive control produced complete larval mortality, confirming the validity of the bioassay. Overall, the results demonstrated a clear concentration-dependent increase in larvicidal activity for all active n-hexane extracts (Table 3).

Determination of LC₅₀ and LC₉₀

Probit analysis was performed to estimate the median lethal concentration (LC₅₀) and the 90% lethal concentration (LC₉₀) of the n-hexane crude extracts from the four selected medicinal plant species against mosquito larvae after 24 h of exposure. The estimated LC₅₀ & LC₉₀ values, together with their 95% confidence intervals and probit regression parameters, are presented in Table 4.

Table 4

LC₅₀ & LC₉₀ values (ppm) of n-hexane crude extracts from selected medicinal plants against third-to early fourth-instar larvae of *An. arabiensis*, *An. stephensi*, and *Ae. aegypti* after 24 h of exposure, estimated using probit analysis.

Plant extracts	Mosquito species	LC ₅₀	95% CI		LC ₉₀	95% CI	
			LB	UB		LB	UB
<i>O. gratissimum</i>	<i>An. arabiensis</i>	596.51	495.07	727.74	1084.48	911.641	1387.98
	<i>Ae. Aegypti</i>	1100.78	568.31	2672.25	1727.75	1177.30	5905.19
<i>A. mexicana</i>	<i>An. arabiensis</i>	650.88	601.26	707.71	1039.50	956.76	1145.76
	<i>An. stephensi</i>	1244.77	971.99	1724.46	1791.20	1425.07	2731.35
	<i>Ae. aegypti</i>	768.00	723.47	812.96	995.17	941.60	1062.39
<i>L. adoensis</i>	<i>An. arabiensis</i>	1261.32	871.60	2023.84	2060.39	1527.65	3811.63
	<i>An. stephensi</i>	860.60	811.09	914.33	1165.88	1093.08	1263.65
	<i>Ae. aegypti</i>	763.03	622.52	973.26	1158.01	953.81	1602.61
<i>C. aurea</i>	<i>An. arabiensis</i>	818.73	768.29	874.75	1153.17	1076.24	1254.02
	<i>Ae. aegypti</i>	1379.26	745.08	3962.58	2186.78	1484.38	9358.13

Note: LC₅₀ = lethal concentration causing 50% mortality of exposed larvae.

LC₉₀ = lethal concentration causing 90% mortality. LB = lower confidence limit; UB = upper confidence limit.

Adulticidal Test

Adulticidal activity of the crude solvent extracts was evaluated using the CDC bottle bioassay method, following established protocols [40, 41, 42]. Plant extracts were dissolved in acetone to prepare the test concentrations. Acetone served as the negative control, while no synthetic insecticide was used as a positive control. The n-hexane and 80% methanol crude extracts of the selected plant species were screened at concentrations ranging from 62.5–2000 ppm.

Clean and dry 250 mL Wheaton bottles with screw caps were coated with 1 mL of each extract solution by swirling to ensure complete coverage of the inner surface and cap. Control bottles were treated similarly with 1 mL acetone. Twenty-five adult mosquitoes (2–5 days old), previously fed on 10% sucrose solution, were introduced into each bottle using a mouth aspirator. After 2 h exposure, mosquitoes were transferred into 250 mL paper cups and provided with 10% sucrose solution. Mortality was recorded after 24 h.

Table 5

Adult mortality (%) of *An. arabiensis* and *Ae. aegypti* exposed to n-hexane crude extracts after 24 hours exposure.

Plants	Mosquito Species	Conc. (PPM)	Mean %	MD	95% CI		Sig.
					LB	UP	
		Acetone	0.00	-100.0	-118.2	-81.77	1.00
<i>L. sativum</i>	<i>An. arabiensis</i>	2000	100.00	-100.0	-93.12	-76.37	.000
		1000	84.67	-84.67	-55.52	-38.77	.000
		500	47.15	-47.15	-15.12	1.62	.000
		250	6.75	-6.75	-8.705	8.03	.001
		125	.333	-.333	-8.37	8.37	.196
		62.5	0	0.00	0.00	3.70	1.00
	<i>Ae. aegypti</i>	2000	96.03	-96.03	-108.32	-86.33	.000
		1000	88.92	-88.92	-99.99	-78.00	.000
		500	61.93	-61.93	-73.32	-51.34	.000
		250	2.00	-2.00	-12.99	8.99	.992
		125	0.00	.00	-10.99	10.99	1.00
		62.5	0.00	.00	-11.24	11.24	1.00
<i>O. lamiifolium</i>	<i>An. arabiensis</i>	2000	100.00	-100.00	-106.56	-93.43	.000
		1000	92.80	-92.80	-99.56	-86.43	.000
		500	16.00	-16.00	-23.56	-10.43	.000
		250	9.20	-9.20	-16.89	-3.77	.000
		125	6.00	-6.00	-12.56	5.56	.099
		62.5	3.40	-3.40	-8.89	4.22	.955
	<i>Ae. aegypti</i>	2000	100.00	-100.00	-102.30	-97.69	.000
		1000	89.00	-89.00	-93.30	-88.69	.000
		500	8.00	-8.00	-10.30	-5.69	.000
		250	.00	.00	0.00	3.70	1.00
		125	.00	.00	0.00	3.70	1.00

Note: Values represent the mean of triplicate bioassays.

Plants	Mosquito Species	Conc. (PPM)	Mean %	MD	95% CI		Sig.
					LB	UP	
		62.5	.00	.00	0.00	3.70	1.00
<i>A. hybridus</i>	<i>An. arabiensis</i>	2000	44.00	-44.00	-51.43	-36.56	.000
		1000	26.00	-26.00	-33.43	-18.56	.000
		500	8.00	-8.00	-15.43	- .56	.027
		250	2.00	-2.00	-9.43	5.43	.866
		125	.00	.00	-7.432	7.432	1.00
		62.5	.00	.00	-7.43	7.43	1.00
	<i>Ae. aegypti</i>	2000	100.00	-100.00	104.50	-95.49	.000
		1000	82.93	-82.93	-87.83	-78.83	.000
		500	30.29	-30.29	-33.83	-24.83	.000
		250	3.41	-3.41	-6.50	2.50	.866
		125	.00	.00	-4.50	4.50	1.00
		62.5	.00	.00	-4.50	4.51	1.00
<i>P. schimperi</i>	<i>An. arabiensis</i>	2000	100.00	-100.00	-116.21	-82.45	.000
		1000	85.00	-85.00	-101.88	-68.11	.000
		500	34.00	-34.33	-50.88	-17.11	.000
		250	2.00	.00	-18.88	14.88	1.00
		125	.00	.00	-16.884	16.88	1.00
		62.5	.00	.00	-16.88	16.88	1.00
	<i>Ae. aegypti</i>	2000	62.08	-62.08	-74.917	-50.34	.000
		1000	37.54	-37.65	-49.833	-25.25	.000
		500	6.454	-6.45	-5.833	18.74	.988
		250	.00	.00	-5.833	18.74	1.00
		125	.00	.00	-5.833	18.74	1.00
		62.5	.00	.00	-5.833	18.74	1.00
<i>A. mexicana</i>	<i>An. arabiensis</i>	2000	100.00	-100.00	-111.94	-88.05	.000
Note: Values represent the mean of triplicate bioassays.							

Plants	Mosquito Species	Conc. (PPM)	Mean %	MD	95% CI		Sig.		
					LB	UP			
	Ae. aegypti	1000	53.67	-53.67	-65.60	-41.72	.000		
		500	21.00	-21.00	-32.941	-9.05	.000		
		250	.00	.00	-11.941	11.94	1.00		
		125	.00	.00	-11.941	11.94	1.00		
		62.5	.00	.00	-11.941	11.94	1.00		
		2000	100.00	-100.0	-104.24	-95.75	.000		
		1000	63.25	-63.25	-67.49	-59.00	.000		
		500	6.00	-6.00	-10.24	-1.75	.001		
		250	.00	.00	-4.24	4.24	1.00		
		125	.00	.00	-4.24	4.24	1.00		
		62.5	.00	.00	-4.24	4.24	1.00		
		L. adoensis	An. arabiensis	2000	49.75	-49.75	-53.53	-45.96	.000
				1000	5.00	-5.00	-8.78	-1.214	.003
				500	.00	.00	-3.78	3.78	1.00
250	.00			.00	-3.786	3.78	1.00		
125	.00			.00	-3.78	3.78	1.00		
62.5	.00			.00	-3.78	3.76	1.00		
Ae. aegypti	2000		100.00	-100.00	-105.76	-94.23	.000		
	1000		65.67	-65.33	-71.43	-59.89	.000		
	500		3.33	-3.33	-9.10	2.43	.586		
	250		1.00	-1.00	-6.76	4.76	.866		
	125		.00	.00	-5.76	5.76	1.00		
	62.5		.00	.00	-5.76	5.76	1.00		
C. aurea	An. arabiensis	2000	100.00	-100.0	-103.29	-96.70	.000		
		1000	73.25	-73.25	-76.54	-69.95	.000		
		500	7.33	-7.33	-10.62	-4.03	.000		
Note: Values represent the mean of triplicate bioassays.									

Plants	Mosquito Species	Conc. (PPM)	Mean %	MD	95% CI		Sig.
					LB	UP	
		250	.00	.00	-3.29	3.29	1.00
		125	.00	.00	-3.29	3.29	1.00
		62.5	.00	.00	-3.29	3.29	1.00
	<i>Ae. aegypti</i>	2000	74.16	-74.16	-77.90	-70.42	.000
		1000	62.41	-62.41	-66.15	-58.67	.000
		500	2.00	-2.00	-5.73	1.73	.670
		250	.00	.00	-3.73	3.73	1.00
		125	.00	.00	-3.73	3.73	1.00
		62.5	.00	.00	-3.73	3.73	1.00
Note: Values represent the mean of triplicate bioassays.							

The adulticidal activity of crude extracts from seven medicinal plant species was evaluated against adult *An. arabiensis* and *Ae. aegypti*. Preliminary screening showed that only the n-hexane crude extracts of *O. lamiifolium*, *L. sativum*, *A. hybridus*, *P. schimperi*, *A. mexicana*, *L. adoensis*, and *C. aurea* produced > 40% mortality after 24 h of exposure. These extracts were therefore selected for concentration–response bioassays (62.5–2000 ppm), whereas all 80% methanol extracts produced < 40% mortality and were excluded from further evaluation.

The adulticidal responses of the selected n-hexane extracts are summarized in Table 5.

All extracts exhibited significant concentration-dependent activity against *An. arabiensis* and *Ae. aegypti*, with mortality increasing progressively as extract concentration increased. Significant differences in adult mortality were observed at concentrations ≥ 500 ppm compared with the negative control ($P < 0.05$), while mortality at lower concentrations (< 500 ppm) did not differ significantly from the acetone control ($P > 0.05$).

Among the tested extracts, *O. lamiifolium* and *A. mexicana* were the most potent, producing significantly higher mortality than the remaining extracts ($P < 0.001$). At 2000 ppm, both extracts achieved complete (100%) mortality against *An. arabiensis* and *Ae. aegypti*. Likewise, *L. adoensis* and *A. hybridus* caused complete mortality of *Ae. aegypti* at the same concentration, whereas *L. sativum*, *P. schimperi*, and *C. aurea* exhibited moderate to high adulticidal activity. No mortality was observed in the acetone-treated control throughout the study, confirming that the recorded mortality resulted from exposure to the plant extracts. Overall, the findings demonstrate that the adulticidal efficacy of the n-hexane crude extracts was strongly concentration dependent, with *O. lamiifolium* and *A. mexicana* showing the greatest potential as botanical adulticidal.

Determination of LC₅₀ and LC₉₀

Probit regression analysis was used to estimate the median lethal concentration (LC₅₀) and the 90% lethal concentration (LC₉₀) of the active n-hexane crude extracts against adult *A. arabiensis* and *A. aegypti* after 24 h of exposure. The estimated LC₅₀ and LC₉₀ values, together with the corresponding 95% confidence intervals are presented in Table 6.

Table 6

Probit-derived LC₅₀ and LC₉₀ values (ppm) of n-hexane crude extracts against adult *An. arabiensis* and *Ae. aegypti* after 24 h of exposure.

Plants	Mosquito species	LC ₅₀	95% CI		LC ₉₀	95% CI	
			LB	UB		LB	UB
<i>L. sativum</i>	<i>An. arabiensis</i>	325.2	297.0	373.4	563.9	466.5	769.3
	<i>Ae. aegypti</i>	595.26	494.90	5296.60	1222.58	815.50	1222.30
<i>O. lamiifolium</i>	<i>An. arabiensis</i>	338.9	229.9	735.1	2141.5	900.0	28737.8
	<i>Ae. aegypti</i>	594.5	494.9	5296.6	949.2	815.5	1222.3
<i>A. hybridus</i>	<i>An. arabiensis</i>	665.7	593.3	779.7	1528.1	1207.6	2152.4
	<i>Ae. aegypti</i>	524.2	409.0	867.9	1212.2	765.1	3170.2
<i>P. schimperi</i>	<i>An. arabiensis</i>	609.30	543.15	682.40	1060.42	918.25	1248.10
	<i>Ae. aegypti</i>	742.7	651.6	913.0	1525.2	1169.0	2372.9
<i>A. mexicana</i>	<i>An. arabiensis</i>	399.4	338.5	451.6	537.8	473.5	676.46
	<i>Ae. aegypti</i>	1147.8	946.7	1534.0	3131.3	2174.4	5540.2
<i>L. adoensis</i>	<i>An. arabiensis</i>	2842.4	2084.4	4574.4	12787.2	7197.9	31532.8
	<i>Ae. aegypti</i>	1359.0	985.1	2449.4	4269.9	2387.3	12684.9
<i>C. aurea</i>	<i>An. arabiensis</i>	701.2	623.9	836.0	1430.6	1124.0	2102.3
	<i>Ae. aegypti</i>	1462.2	975.0	4445.1	3747.9	1858.2	26639.5

Note

LC₅₀ = lethal concentration causing 50% mortality of exposed adult mosquito; LC₉₀ = lethal concentration causing 90% mortality. LB = lower confidence limit; UB = upper confidence limit.

DISCUSSION

The global imperative to identify sustainable botanical bioinsecticides is driven by accelerating environmental degradation and the widespread development of resistance among mosquito populations

to conventional synthetic insecticides. Plant-derived insecticides offer several ecological advantages because they are biodegradable, leave minimal environmental residues, and generally exhibit lower toxicity toward humans and non-target organisms than conventional chemical insecticides [8, 9, 10, 11]. The present study evaluated the larvicidal and adulticidal activities of crude extracts prepared with 80% methanol and n-hexane from eight Ethiopian medicinal plants (*O. lamiifolium*, *L. sativum*, *A. hybridus*, *P. schimperi*, *O. gratissimum*, *A. mexicana*, *L. adoensis*, and *C. aurea*) against *An. arabiensis*, *Ae. aegypti*, and *An. stephensi*.

Our laboratory bioassays revealed a clear solvent-dependent difference in bioinsecticidal activity. The 80% methanol extracts exhibited negligible or no larvicidal and adulticidal activity, whereas the n-hexane extracts showed moderate to very high toxicity against the tested mosquito species. These findings indicate that the major insecticidal constituents are predominantly lipophilic and are therefore extracted more efficiently by non-polar solvents. Solvent polarity is well known to influence both the extraction efficiency and biological activity of plant secondary metabolites, and several previous studies have similarly reported greater insecticidal efficacy for non-polar extracts than for polar extracts [14, 42, 43, 44, 45].

A notable finding of the present study is the comparative performance of the two *Ocimum* species. Although *O. lamiifolium* was previously investigated mainly for adulticidal activity and reported separately for larvicidal efficacy, the inclusion of *O. gratissimum* enabled direct comparison between two closely related Ethiopian medicinal plants. Species of *Ocimum* are rich in volatile terpenoids and phenylpropanoids, including eugenol, β -caryophyllene, thymol, and γ -terpinene, compounds that possess well-documented insecticidal and repellent activities [11, 12, 13, 14, 39]. The stronger larvicidal activity observed for *O. gratissimum*, reflected by its lower LC_{50} and LC_{90} values against *An. arabiensis*, may therefore be attributed to differences in essential-oil composition and the synergistic action of these lipophilic constituents.

The superiority of n-hexane extracts also emphasizes the importance of solvent selection during phytochemical extraction. Extraction solvent determines not only the quantity of recovered metabolites but also their chemical composition and biological activity. Previous studies have demonstrated that the polarity of extraction solvents strongly affects the recovery of terpenoids, alkaloids, flavonoids, and other bioactive metabolites responsible for insecticidal activity [14, 42, 43, 44, 45]. Our findings therefore agree with earlier reports showing that decreasing solvent polarity generally increases the toxicity of botanical extracts against mosquito vectors.

The strong activity observed for *O. gratissimum*, *A. mexicana*, *L. adoensis*, and *C. aurea* against mosquito larvae corroborates previous reports describing the insecticidal potential of these medicinal plants and related species [11, 20, 22, 23, 24, 46, 47, 48]. In particular, *O. gratissimum* and *A. mexicana* produced complete larval mortality against *An. arabiensis* at the highest concentration tested, whereas *A. mexicana* and *L. adoensis* also achieved nearly complete mortality against *Ae. aegypti* and *An. stephensi*.

Such activity suggests the presence of potent bioactive metabolites capable of disrupting essential physiological processes in mosquito larvae.

Similarly, the adulticidal activity demonstrated by *O. lamiifolium*, *L. sativum*, *P. schimperi*, and *A. mexicana* supports their traditional use in Ethiopian ethnomedicine for insect control [11, 15, 16, 18, 19, 47, 48, 49, 50, 51, 52, 53]. These plants contain diverse phytochemicals—including alkaloids, flavonoids, tannins, phenolics, terpenoids, and essential oils—that have previously been associated with insecticidal, repellent, and antimicrobial activities [11, 16, 47, 48]. These compounds may exert toxicity through disruption of the insect nervous system, impairment of respiratory function, inhibition of feeding, or damage to the larval midgut and osmoregulatory tissues [11, 20, 22, 23, 46, 47, 48].

Differences in bioinsecticidal activity among plant species and mosquito vectors observed in this study are likely attributable to multiple interacting factors, including phytochemical composition, solvent polarity, extraction method, plant genotype, environmental conditions during plant growth, and mosquito susceptibility [11, 15, 16, 18, 19, 47, 48, 49, 50, 51, 52, 53].

Environmental factors such as soil characteristics, light intensity, rainfall, altitude, and temperature are known to influence the accumulation of secondary metabolites and consequently affect biological activity [14, 49, 50, 51, 52, 53].

Overall, the present findings demonstrate that n-hexane extracts consistently outperformed methanol extracts, confirming that non-polar phytochemicals are primarily responsible for the observed mosquito mortality. Among the evaluated plants, *O. gratissimum* exhibited the greatest larvicidal potency, whereas *L. sativum* showed particularly strong adulticidal activity against *An. arabiensis*. These results highlight the potential of Ethiopian medicinal plants as promising sources of environmentally friendly botanical bioinsecticides and support further bioassay-guided isolation, chemical characterization, and field evaluation of their active constituents.

CONCLUSION

This study demonstrated that the n-hexane crude extracts of the selected medicinal plants possess promising larvicidal and adulticidal activities against *An. arabiensis*, *Ae. aegypti*, and *An. stephensi*, whereas the 80% methanol extracts showed limited activity. Among the tested plants, *A. mexicana* exhibited outstanding larvicidal efficacy against all three mosquito species, while *L. adoensis* achieved nearly 100% larval mortality against *Ae. aegypti* and *An. stephensi* at 2000 ppm. In addition, seven n-hexane crude extracts demonstrated concentration-dependent adulticidal activity against *An. arabiensis* and *Ae. aegypti*, highlighting their potential as sources of botanical bioinsecticides. These findings support further bioassay-guided isolation and characterization of the active phytochemicals, together with semi-field and field evaluations, to facilitate the development of effective, environmentally friendly mosquito control agents.

Limitations

This study has several limitations. First, the efficacy of the plant extracts was evaluated only under laboratory conditions and was not validated under semi-field or field conditions, or against all developmental stages of the target mosquito vectors. Second, bioassay-guided phytochemical investigations were not conducted to isolate and characterize the active insecticidal compounds because of financial constraints.

Abbreviations

ANOVA Analysis of variance

IRS Indoor residual insecticide spraying

LLINs Long-lasting insecticide-treated mosquito nets

SPSS Statistical Package for the Social Sciences

LC Lethal concentration

Declarations

Acknowledgements

The authors sincerely thank Mr. Melaku Wondafirash for his assistance with the identification and preservation of the plant specimens.

Authors' Contributions

N.G. conceived and designed the study, conducted the experiments, performed data analysis, and drafted the manuscript. S.D. contributed to the study conception and design, secured funding, and critically revised the manuscript. K.T. contributed to the study design, obtained funding, supervised the research, processed and extracted the plant materials, prepared stock and serial dilution solutions, and revised the manuscript. A.G. contributed to the study design and manuscript preparation. E.A. participated in the study design and critically reviewed the manuscript. B.K. and D.B. contributed to the study design and implementation. All authors reviewed, approved, and agreed to the final version of the manuscript.

Funding

This work was supported by the Office of the Vice President for Research and Technology Transfer, Addis Ababa University.

Data Availability

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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. Benelli G, Duggan MF. Management of arthropod vector data-Social and ecological dynamics facing the One Health perspective. *Acta tropica*. 2018; 182:80–91. doi: 10.1016/j.actatropica.2018.02.015.
2. Krauer F, Riesen M, Reveiz L, Oladapo OT, Martínez-Vega R, Porgo TV, et al. Zika virus infection as a cause of congenital brain abnormalities and Guillain-Barré Syndrome: systematic review. *PLoS medicine*. 2017; 14:e1002203. doi: 10.1371/journal.pmed.1002203.
3. Becker N, Petrić D, Zgomba M, Boase C, Madon M, Dahl C, et al. Subfamily anopheline. In: Mosquitoes & Their control. New York, USA: Springer. 2010; 163 – 86.
4. WHO. Dengue and severe dengue. Geneva, Switzerland. 2020. Available at: <https://www.who.int/news-room/fact-sheets/detail/dengue-and-severe-dengue> (Accessed November 13, 2022).
5. Al Zahrani MR, Gharsan FN, Al-Ghamd KM, Mahyoub JA, Alghamdi TS. Toxicity of two groups of pesticides against the mosquito *Aedes aegypti*. *GSC Biol Pharmacol Sci*. (2020) 13:148–55. doi: 10.30574/gscbps.2020.13.1.0334
6. Junkum A, Intirach J, Chansang A, Champakaew D, Chaithong U, Jitpakdi A, et al. Enhancement of temephos and deltamethrin toxicity by *Petroselinum crispum* oil and its main constituents against *Aedes aegypti* (diptera: culicidae). *Med Entomol*. 2021; 58:1298–315. doi: 10.1093/jme/tjab008.
7. 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 Neg Tropic Dis*. 2021; 15:e0009036. doi: 10.1371/journal.pntd.0009036.
8. 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 Bull*. 2004; 28:184–8.

9. Uwaifo F, John-Ohimai F. Dangers of organophosphate pesticide exposure to human health. *Mat Sci Med.*2020; 4:27. doi: 10.4103/MTSM.MTSM_2_20.
10. Pratiwi MAM, Purwati. The repellent activity test of rosemary leaf (*Rosmarinus officinalis* 1) essential oil gel preparations influence on *Aedes aegypti* mosquito. *J. Phys.Conf. Ser.* 2021; 1788, 012016. doi: 10.1088/1742-6596/1788/1/012016
11. Govindarajan M, Rajeswary M, Hoti S L and 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), *Res Vet Sci*, 2016, 104, 77–82.
12. Kačániová M, Galovičová L, Borotová P, Vukovic NL, Vukic M, Kunová S, et al. Assessment of *Ocimum basilicum* essential oil anti-insect activity and antimicrobial protection in fruit and vegetable quality. *Plants.* 2022; 11:1030.
13. Sahalie NA, Abrha LH, Tolesa LD. Chemical composition and antimicrobial activity of leave extract of *Ocimum lamiifolium* (Damakese) as a treatment for urinary tract infection. *Cogent Chem.* 2018; 4(1):1440894. <https://doi.org/10.1080/23312009.2018.1440894>.
14. Yang L, Wen KS, Ruan X, Zhao YX, Wei F, Wang Q. A review response of plant secondary metabolites to environmental factors. *Molecules.* 2018; 23(4):762.
15. Moges A, Moges Y. Important Medicinal plants of Ethiopia: uses, knowledge transfer and conservation practices. 2021; ©Encyclopedia of Life Support Systems (EOLSS).
16. Overgaard HJ, Sirisopa P, Mikolo B, Malternd KE, Wangenstein H, Zou Y, et al. Insecticidal activities of bark, leaf and seed extracts of *Zanthoxy heitzii* against the African malaria vector *Anopheles gambiae*. *Molecules.* 2014; 19:21276–90.
17. Chowdhury JU, Nandi NC, Bhuiyan NI. Chemical composition of leaf essential oil of *Lan/ana camara* L. *Bangladesh J Bot.* 2007; 36:193–4.
18. Abebe D, Debellia A, Urga K. Checklist of medicinal plants and other useful plants of Ethiopia. *Camerapix pub, pvt. Ltd. Co: Singapore*; 2003. p. 312.
19. Berhanu A, Asfaw Z, Kelbessa E. Ethno-botany of plants used as insecticides, repellents and anti-malarial agents in Jabitehnan district, West Gojjam. *SINET Ethiop Sci.* 2006; 29(1):87–92.
20. Ghosh A, Chowdhury N, Chandra G. Plant extracts as potential mosquito larvicides. *Indian J Med Res.* 2012; 135:581.
21. 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; 16:275–8.
22. Chac LD, Toan NV, Son PH, Thinh BB. Chemical composition and antimicrobial activity of the essential oil from *premna interrupta*. *Chem Nat Compd.* 2025. <https://doi.org/10.1007/s10600-025-04765-5>.
23. Hung NH, Huong LT, Chung NT, Truong NC, Dai DN, Satyal P, et al. *Premna* species in vietnam: essential oil compositions and mosquito larvicidal activities. <https://doi.org/10.3390/plants9091>

24. Balcha Abera. Medicinal plants used in traditional medicine by Oromo people, Ghimbi District, Southwest Ethiopia. 2014, 10:40. <http://www.ethnobiomed.com/content/10/1/40>.
25. Chore JK, Obonyo M, Wachira FN, Mireji PO. Larvicidal activity of selected *Aloe* species against *Aedes aegypti* (Diptera: Culiciade). *J Insect Sci.* 2014; 14:202.
26. Chinnaperumal Kamaraj and Abdul Rahuman. Larvicidal and adulticidal potential of medicinal plant extracts from south India against vectors. 2010, 948–953; journal homepage: www.elsevier.com/locate/apjtm.
27. Dr. Monika Sanyal. Phytochemical Profiling of Crude Extracts of Leaves of *Argemone Mexicana*. *International Journal of Research Publication and Reviews*. March 2023, no 3, pp 4966–4968, Journal homepage: www.ijrpr.com ISSN 2582–7421.
28. Afroz Alam. *Argemone mexicana* L.: A weed with versatile medicinal and pharmacological applications. June, 2020. DOI: 10.21276/ap.2020.9.1.29: <https://www.researchgate.net/publication/342676533>.
29. Mirunaliniraj ant Sundararajan. Effect of *Argemone Mexicana* plant extracts on larval mortality of *Helicoverpa armigera* (Hubner). *International Journal of Entomology Research*, 2025; Page No. 42–45, www.entomologyjournals.com
30. Yordanos Germame and Tegenu Mekuria. Identification of Bioactive Compound, Phytochemical Analysis and Antimicrobial Activity of *Lippia adoensis* var. *koseret* from Ethiopia. 20218; DOI: 10.36348/sijtcm.2021.v04i08.001.
31. Fekadu Massebo¹, Mekuria Tadesse, Meshesha Balkew, Teshome Gebre-Michael. Bioactivity of essential oils of local plants against adult *Anopheles arabiensis* (Diptera: Culicidae) in Ethiopia. 2013, 4, 805809. <http://dx.doi.org/10.4236/abb.2013.48105>. (<http://www.scirp.org/journal/abb/>)
32. Sileshi Degu, Asfaw Berihun, Rekik Muluye, Hirut Gemed, Eyob Debebe, Aliyi Amano, Abiy Abebe, Samuel Woldkidan, Ashenef Tadele, Review Article. Medicinal plants that used as repellent, insecticide and larvicide in Ethiopia. *Pharmacy & Pharmacology International Journal*. September 10, 2020; Open Access. Cross mark.
33. Muhammed et al. Insecticidal effects of some selected plant extracts against *Anopheles stephensi* (Culicidae: Diptera). *Malaria Journal*. 2022, 21:295. <https://doi.org/10.1186/s12936-022-04320-5>.
34. Kudom AA, Mensah BA, Botchey MA. Aqueous neem. Extract versus neem powder on *Cu/ex quinquefasciatus*: implications for control in anthropogenic habitats. *J Insect Sci.* 2011; 11:142.
35. Zorlonia, c, B.L. Penzhornb and J.N. Eloffa Extracts of *Calpurnia aurea* leaves from southern Ethiopia attract and immobilise or kill ticks. Via Sempione 10, 28868 Varzo, Italy.
36. WHO. Guidelines for laboratory and field testing of mosquito larvicides. Geneva, World Health Organization; 2005b.
37. Abbott WS. A method of computing the effectiveness of an insecticide *Econ Entomol.* 1925; 18: 265–267.32. SPSS (2007) SPSS for windows, version 16.0.

38. Balkew M, Mumba P, Yohannes G, Abiy E, Getachew D, Yared S, et al. An update on the distribution, bionomics, and insecticide susceptibility of *Anopheles stephensi* in Ethiopia, 2018–2020. *Malar J*. 2021; 20:263.
39. Negesse Gebissa, Ketema Tolossa, Araya Gebresilassie, Esayas Aklilu, Daniel Bisrat, Bersissa Kumsa and Sisay Dugassa. Larvicidal effects of selected medicinal plant extracts against *An. arabiensis*, *An. stephensi*, and *Ae. aegypti*. 2025, 53:197; <https://doi.org/10.1186/s41182-025-00879-2>.
40. Brogdon WG, McAllister JC. Simplification of adult mosquito bioassays through use of time-mortality determinations in glass bottles. *J Am Mosq Control Assoc*. 1998; 14:159–64.
41. CDC. Centers for Disease Control and Prevention. Guideline for evaluating insecticide resistance in vectors using the CDC bottle bioassay. 2010.
42. Shaalan EA, Canyon D, Younes MWF, Abdel-Wahab H, Mansour A. A review of botanical phytochemicals with mosquitocidal potential. *Environ Int*. 2005; 31:1149–66.
43. Aivazi AA, and Vijayan VA. Larvicidal activity of oak *Quercus infectoria* oliv. (Fagaceae) gall extracts against *Anopheles stephensi* Liston. *Parasitol Res*. 2009; 104:1289–93.
44. Mulla MS, Su T. Activity and biological effects of neem products against arthropods of medical and veterinary importance. *Jam Mosq Cont Assoc*. 1999; 15:133–52.
45. Hidayatulfathi O, Sallehuddin S, Ibrahim J. Adulticidal activity of some Malaysian plant extracts against *An. aegypti*. *Trap Biomed*. 2004; 21(2):61–7.
46. Abubakar N, Mukhtar MM, Fujita R. Telfairia occidentalis as a potential larvicide against human malarial vector, *Anopheles coluzzi* [Diptera: Culicidae]: A first report. *Int J Trop Dis*. 2022, 5(1).
47. Pavithra KH, Narase Gowda PN, Prasad MP. Studies on antimicrobial activity of *Ocimum* species of Karnataka against clinical isolates. *Int J Pure Appl Biosci*. 2019; 7:245–54.
48. Joshi B, Lekhak S, Sharma A. Antibacterial Property of Different Medicinal Plants: *O. sanctum*, *C. zeylanicum*, *X. armatum* and *O. majorana*. Kathmandu Univ J Sci Eng Technol. 2009; 5:143–50.
49. Perumalsamy H, Kim J-R, Oh SM, Jung JW, Ahn Y-J, et al. Novel histopathological and molecular effects of natural compound pellitorine on larval midgut epithelium and anal gills of *Ae. aegypti*. *PLoS ONE*. 2013; 8:e80226.
50. Costa MS, Cossolin JF, Pereira MJ, Santana AE, Lima MD, et al. Larvicidal and cytotoxic potential of squamocin on the midgut of *Aedes aegypti* (Diptera: Culicidae). *Toxins*. 2014; 6:1169–76.
51. Aydin T, Bayrak N, Baran E, Cakir. Insecticidal effects of extracts of *Humulus lupulus* (hops) L. cones and its principal component, xanthohumol. *Bull Entomol Res*. 2017; 107:543.
52. Rajkumar S, Jebanesan A. Oviposition deterrent and skin repellent activities of *Solanum trilobatum* leaf extract against the malaria vector *An. stephensi*. *J Insect Sci*. 2005; 5:15.
53. Procopio TF, Fernandes KM, Pontual EV, Ximenes RM, De Oliveira ARC, et al. *Schinus terebinthifolius* leaf extract causes midgut damage, interfering with survival and development of *Ae. aegypti* larvae. *PLoS ONE*. 2015; 10:e0126612.