

Larvicidal effects of Selected Medicinal Plant Extracts against *Anopheles arabiensis*, *Anopheles stephensi*, and *Aedes aegypti*

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Abstract

ABSTRACT Background: The emergence of resistance, along with their harmful effects on human health, non-target organisms, and the environment, of synthetic insecticides necessitate the development new complementary insecticides that are effective, environmentally friendly, biodegradable, and target-specific. This study was undertaken to evaluate larvicidal activities of 80% methanol and n-hexane extracts of four plants that are traditionally used by communities against mosquitoes. Methods: The dried leaves of *Ocimum lamiifolium*, *Amaranthus hybridus* and *Premna schimperi*, and seeds of *Lepidium sativum* were extracted with 80% methanol and n-hexane solvents. Larvicidal activities of these extracts were evaluated under laboratory condition in the range of 62.5 to 2000 ppm against the late 3rd - early 4th instar larvae of *An. arabiensis*, *An. stephensi* and *Ae. Aegypti* mosquitoes. Larvae mortality was observed after 24 h of exposure. The mortality data were subjected to probit analysis to determine LC50 and LC90 values. Results: At 2000 ppm concentration, the n-hexane extract of the tested plants: *Ocimum lamiifolium* against *An. arabiensis*, *An. stephensi* and *Ae. aegypti*, and *Amaranthus hybridus* against *An. stephensi* and *Ae. aegypti*, *Lepidium sativum* and *Premna schimperi* against *An. Arabiensis* exhibited 100%, larvicidal activity with LC50 and LC90 values of 666.07, 1278.22, 713.25 & 1920.82, 2139.91 & 988.90; 874.78, 636.761 and 1426.03, 1122.94; 412 & 1222.62, and 736.150 & 1120.67 respectively. All the n-hexane extracts showed larvicidal activity whereas, all the 80% methanol crude extracts were not selected and subjected to dose-response bio-assay, yielding the mortality of larvae < 40% after 24 h during the preliminary screening at 2000 ppm. Conclusion: The n-hexane crude extracts of the tested plants have the potential to be used as larvicides against larvae of *An. arabiensis*, *An. stephensi* and *Ae. Aegypti*. Therefore, it is necessary to undertake studies that focused on bioassay-guided isolation, purification & structural elucidation of active compound (s) from the most active n-hexane fractions of tested plants to develop a product that complement the current existing vector control tools.

Background

Mosquito-borne diseases (MBDs) are increasingly prevalent due to the resultant impact of global change with significant health and economic impacts worldwide [1]. *Anopheles* and *Aedes* mosquitoes are among the most important groups of arthropods with medical significance that transmit several important parasitic and arboviral diseases [2]. Malaria, a life-threatening disease, is primarily transmitted to humans through the bites of infected female *Anopheles* mosquitoes, including *An. arabiensis* and *An. stephensi* [3]. *An. arabiensis* is also a major malaria vector within the *An. gambiae* species complex in sub-Saharan Africa and surrounding islands [4].

Arboviruses continue to generate significant health and economic burdens for people living in endemic regions such as East Africa including Ethiopia [5]. These viruses (dengue, Zika, chikungunya, and yellow fever virus) are transmitted mainly by *Aedes* mosquitoes [6]. Arbovirus infections are a global public health threat, accounting for approximately 73% of the total emerging and re-emerging human infections, where the burden is worsened in sub-Saharan Africa, including Ethiopia [7]. Malaria, caused by protozoans and arboviral diseases caused by viruses can lead to high mortality and morbidity in tropical and sub-tropical regions [8]. The spread of these mosquito vectors in different parts of East African Region including Ethiopia has become a serious concern for malaria and arboviral diseases prevention and elimination strategies [9]. Global malaria cases and deaths have been significantly reduced following the scaling up of long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) [10]. However, widespread use of synthetic insecticides in controlling mosquito vectors has resulted in the persistence and accumulation of non-biodegradable chemicals in the ecosystem, development of resistance to insecticides in vectors, and toxic effects in non-target organisms including humans [11].

As evidenced in recent studies from different parts of Ethiopia, all the three mosquito species have shown resistance to insecticides belonging to the four chemical classes (such as organochlorines, organophosphates, carbamates, and pyrethroids) and approved for IRS and ITNs [12]. There is evidence of changes in biting and resting behavior of the main malaria vectors as a result of selective pressures by the widespread and long-term use of bed nets and indoor residual spraying [13]. The emergence of insecticide resistance necessitates an urgent need to develop natural product-based mosquito control methods that are biodegradable, economical, effective and less toxic to non-target organisms including edible aquatic animals, humans and the environment [14]. In this regard, plant extracts with insecticidal potential are recognized as complementary and/ alternative plant-based products to synthetic insecticides in mosquito control programs due to their ovicidal, larvicidal, adulticidal and repellence

properties that may enhance the discovery of botanical-based products that are safe, biodegradable, and target specific natural agents [15].

Therefore, in view of the recently increased interest in developing plant-derived larvicides/insecticides, the present study was designed with the objective of evaluating the larvicidal potential of polar (80% methanol) and non-polar (n-hexane) plant extracts against *An. arabiensis*, *An. stephensi*, and *Ae. aegypti* from four different test plant species namely; *Ocimum lamiifolium*, *Lepidium sativum*, *Amaranthus hybridus*, and *Premna schimperi* that are repeatedly used traditionally as mosquito control and reported to be effective against larvae of mosquito vectors of malaria and arboviral diseases in Ethiopia, following the standard WHO bioassay test methods and procedures. Here, the present study results will be used as a prerequisite for future research to develop eco-friendly and efficient alternatives to synthetic larvicides in large-scale mosquito control programs that target vectors of malaria and viral infections under laboratory conditions.

Materials and Methods

Selection and Collection of Plant Species

The plant species that were evaluated for larvicidal properties were collected following the ethno-taxonomic approach by searching targeted plant families from the literature in the manner discussed by [16]. The information on larvicidal plants were collected from published articles, theses and research reports. Different ethno botanical publications by different researchers over the past couple of decades were reviewed. Google Scholar and Pub med were browsed using some important key words such as insecticide, larvicides and medicinal plants. Appropriate data collection format was prepared to tabulate scientific, family and local names of species, plant part used, method of application and the sources of references for each species. In order to select the most relevant and promising species based on the use report for insecticides for the purpose of screening, the candidate test plant species were listed by taking into consideration the fidelity level (Fidelity level is a statistical tool to measure the potentiality of the specific plant for treating the specific disease [17], whereby it enables the scholar to identify the most important species), the citation frequency of medicinal plants (at least in 3 reviewed articles) and the availability of important and adequate information of each of the medicinal plants in the reviewed documents. Accordingly, the candidate plant species were initially compiled from literature followed by field trips.

Four plant species namely *Ocimum lamiifolium*, *Lepidium sativum* L., *Amaranthus hybridus* L. and *Premna schimperi* Engl. were selected, and collected based on their traditional use as larvicides/insecticides. The plant materials both (voucher specimens, and materials for extraction and testing) were collected during field trips at the sites in the months of August and November, 2024, respectively, from Oromia Regional State, Jimma zone, Asendabo district and Southwest Ethiopia Regional State, Kaffa Zone, Bonga district, , Ethiopia.

Voucher Specimen Collection and Identification

The plant species were authenticated by a plant taxonomist at the National Herbarium, College of Natural and Computational Sciences, AAU, Ethiopia. The voucher specimens were deposited at the National Herbarium of Addis Ababa University. A good recorder of the location, habitat and geography of the collection environment, was also properly labeled and kept as summarized in table 1.

S.N	Species name	Family name	Collection Place	Local name	Habitat	GPS coordinates	Altitude
1	<i>Ocimum lamiifolium</i> Hochst. Ex. Walp (LG-01)	Lamiaceae	Asendabo	Damakase (AO)	Home Stead	(07°46'15.155"N) (037°14'03.585"E)	1,761 m
2	<i>Lepidium sativum</i> L. (LG-10)	Lamiaceae	Asendabo	Feto (AO)	Stead	(07°43'20.34"N) (36°18'09.79"E)	1,772m
3	<i>Amaranthus hybridus</i> L. (LG-06)	Amaranthaceae	Bonga	Asangira (AO)	Forest	(07°43'20.34"N) (36°18'09.79"E)	1,772m
4	<i>Premna schimperi</i> Engl. (LG-04)	Lamiaceae	Asendabo	Urgessa (AO)	Wild	(07°46'05.846"N) (037°014'02.226"E)	1,757m

Table 1: A Records of the Four Test Plant Species

Bulk Sample Collection and Processing

The plant parts of *Ocimum lamiifolium* (leaf), *Lepidium sativum* (seeds), *Premna schimperi* (leaf) and *Amaranthus hybridus* (leaf) samples were collected and kept separately in plastic bags, tied with rubber bands and brought to the AAU, Aklilu Lemma Institute of Health Research (ALHR) laboratory. The collected plant materials were air-dried under shade at room temperature ($25 \pm 2^\circ\text{C}$) for 7 days. The dried plant materials were separately powdered using electrical grinder and sieved to get 1-mm particle sizes to improve the subsequent extraction by rendering the sample more homogenous, increasing the surface area and facilitating the penetration of solvent into cells. The powdered plant materials were placed in air-tight plastic bags, labelled and stored in dark box at room temperature until used for crude extract preparation and bioassays.

Preparation of Crude Extracts

Hundred-grams of each finely powdered plant material was separately soaked in a clean 500 ml flask with 80% methanol and n-hexane solvent systems for extraction at a ratio of 1:5 (w/v) at room temperature for 3 days with continuous stirring at 120 rpm using shaker to ensure complete extraction. At the end of extraction, the micelle of each solvent system was separated from marc by filtration using four layered cloths. Afterwards, the crude extracts were centrifuged by HERMLE (Z 383 K) apparatus at 1500rpm/5min for clarification of the filtered sample extracts. Subsequently, the clear filtrates of extracts of both solvent systems were concentrated using rotary evaporator (*DREHSCHIEBR-VAKUUMPUMPE* ROTARY VANE VUUM PUMP) under reduced pressure at 40°C , while the remaining aqueous portions were collected separately in amberoid glass bottles, and then evaporated to dryness using in an oven set at a temperature of 40°C . The dried plant extracts were weighed, the percentage yields determined and stored in amber-colored vials at 4°C until the stock solutions were prepared and used for bioassays.

Rearing of Mosquitos larvae

Larvae of the three mosquito vectors were reared at the insectary of Aklilu Lemma Institute of Health Research, AAU, Ethiopia. The eggs of *An. arabiensis*, *An. stephensi* and *Ae. aegypti* were kept in the tray containing tap water under laboratory condition. After 24 h of incubation, the eggs were observed to hatch out into first instar larvae. Appropriate amount of nutrient (sterilized yeast powder and dog biscuit in 1:1 ratio) were added to enhance the growth of larvae. The late 3rd to early 4th instar larvae were used in the study. The Larvae colonies were maintained at standardized conditions in a separate room in the insectary at $25 \pm 2^\circ\text{C}$ and $60\% \pm 10\%$ RH under 12:12 (light: dark) photoperiod cycle in the insectary to ensure the reliability & reproducibility of data.

Larvicidal Bioassay

The larvicidal bioassay against laboratory-reared mosquitoes were carried out following the standard procedures of WHO larvicidal test method [18]. Twenty ml of 1% (w/v) stock solutions of each extract was prepared by dissolving 200 mg extracts in 20 ml of 5 dimethyl sulfoxide and twofold serial dilutions of the plant extracts test concentrations (2000, 1000, 500, 250, 125 and 62.5 ppm) were prepared. The n-hexane extracts of the four selected plant species parts (namely: *Ocimum lamiifolium*, *Lepidium*

sativum, *Premna schimperi* and *Amaranthus hybridus*) were evaluated against late 3rd to early 4th instar larvae of *An. arabiensis*, *An. stephensi* and *Ae.aegypti* mosquitoes and then subjected to a dose-dependent bioassay to determine LC₅₀ and LC₉₀ values.

For the bioassay, stock solutions of each extract test concentrations of the plant extracts were serially diluted with 5% dimethyl sulfoxide as described in the WHO larvae bioassay protocol [18]. The mixtures were gently stirred to ensure a homogeneous test solution and kept at ambient temperature. Determination of the desired lower concentrations were prepared by serial dilutions based on the dilution principle following the formula $C_1V_1 = C_2V_2$ as described [19].

One hundred fifty larvae of a given species were required to conduct a single dose-response tests; of these, 100 were exposed to the concentration being tested (in 4 replicates each of 25 mosquitos' larvae) plus 25 larvae in the negative control and 25 larvae in the positive control groups. Each assay was repeated three times. At the end of the 24 h, the number of dead larvae was recorded and the percentage mortality was calculated using the formula [20].

The percentage of mortality = (No. of larva dead /No. of larvae)*100

In the first phase of bio-assay, the larvicidal activity of 80% methanol and n-hexane crude extracts of frequently used candidate test plants *O. lamiifolium*, *L. sativum*, *A. hybridus* and *P. schimperi* were screened at 2000 ppm concentration. The mosquitoes' larvae were exposed to 2000 ppm of test concentration and a control to find out the activity of the materials under test. Based on the preliminary screening, four levels of classifications were used to determine efficacy level of extracts: strong mortality> 80%; moderate mortality 80–60%; weak mortality 60–40%; little or no activity mortality< 40%. Hence, extracts yielding the mortality of larvae> 40% were selected and subjected to dose-response bio-assay. Whereas, none larval mortalities were observed in the corresponding negative control, while the corresponding positive control exhibited 100% mortalities of the respective mosquito larvae during the preliminary screening.

Data Analysis

Results were expressed as the mean percent mortality with standard deviations. One-way analysis of variance (ANOVA) using SPSS for windows (version 26) was used to test differences in mean larval mortality rates between crude extracts. Larvicidal activity was considered to be significantly different when 95% confidence limit levels failed to overlap or if *P* value< 0.05. Comparisons between mean percent mortality rates of larvae that were treated with crude extracts, negative and positive controls were made by using Turkey's Post Hoc testing. The mean mortality rates of 12 replicates were taken to determine the percent mortality after 24 h of exposure by using Turkey's Post hoc HSD test. The average larval mortality data were subjected to generalized linear probit model analysis for calculating LC₅₀, LC₉₀ and other statistics at 95% confidence limits of upper confidence limit and lower confidence limit, and Chi-square values were calculated.

Results

Plant Species collected for the larvicidal Tests

The dried crude extracts were weighed and their percentage yield also calculated. The scientific names, solvents used for extraction and yield of crude extracts of the selected plants used for larvicidal bioassay are summarized in table 2.

S.N	Plant species	Wt (%)	
		80% Methanol	n-hexane
1	<i>O. lamiifolium</i> Hochst. ex. Walp.	5.31	4.7
2	<i>Lepidium sativum</i> L.	11.3	8.1
3	<i>Amaranthus hybridus</i> L.	9.7	2.9
4	<i>Premna schimperi</i>	13.3	4.8

Table 2: The percentage yield of 80% methanol and n-hexane crude extracts of targeted plant species evaluated against larvae of *An. arabiensis*, *An. Stephensi*, and *Ae. Aegypti*.

A total of four extracts from four different medicinal plants were tested for larvicidal activity against *An. arabiensis*, *An. stephensi* and *Ae aegypti*. The larvicidal activity of the candidate test plant species of 80% MeOH and n-hexane crude extracts were evaluated. From these, the n-hexane crude extracts of *O. lamiifolium*, *L. sativum*, *A. hybridus* and *P. schimperi* were selected and subjected to dose-response bio-assays at concentrations of 2000, 1000, 500, 250, 125 and 62.5 ppm (Table 3), yielding larvae mortality of >40% after 24 h during the preliminary screening, while all 80% MeOH crude extract treated larvae mortalities failed under 40% classification range in the first phase of bio-assay or during the screening.

The results of larval mortality were obtained from bioassays of the n-hexane crude extracts of *O. lamiifolium*, *L. sativum*, *A. hybridus* and *P. schimperi* against *An. arabiensis*, *An. stephensi* and *Ae aegypti* at different concentrations after 24 h exposure periods are presented in (Table 3 and Figure 1).

All n-hexane crude extracts of test plants showed low, moderate, and high larvicidal activities between 62.5 to 2000 ppm treatments against the late 3rd - early 4th instar larvae of the respective mosquito larvae. Within the same exposure period, larval mortality was not recorded at lower concentrations (≤ 250 ppm) of extracts and the negative control, while the standard (temephos) achieved 100% of larval mortality.

Plant species	Mosquito species	Conc. (ppm)	Mean %	Minimum	Maximum
<i>Lepidium sativum</i> L.	<i>An. arabiensis</i>	2000	100.0*	100.0	100.0
		1000	90.7*	80.3	100.0
		500	41.3*	16.0	100.0
		250	26.7*	8.00	68.0
		125	13.7*	0	48.0
		62.5	1.67*	0	16.0
	<i>Ae. aegypti</i>	2000	97.3*	84.0	100.0
		1000	89.0*	80.0	96.0
		500	62.3*	24.0	92.0
		250	2.0*	0	4.0
		125	-.*	0	.0
		62.5	-.*	0	.0
<i>O. lamiifolium</i> Hochst. ex. Walp.	<i>An. arabiensis</i>	2000	100.0*	100.0	100.0
		1000	93.0*	84.0	100.0
		500	17.0*	8.0	40.0
		250	10.3*	4.0	16.0
		125	6.0*	0	16.0
		62.5	2.3*	0	12.0
	<i>An. stephensi</i>	2000	99.7*	96.00	100.0
		1000	7.0*	0	12.0
		500	.7*	0	4.0
		250	.4*	0	4.0
		125	-.*	0	0
		62.5	-.*	0	0
	<i>Ae. aegypti</i>	2000	100.0*	100.0	100.0
		1000	91.0*	84.0	96.0
		500	8.0*	0	12.0
		250	-.*	0	0
		125	-.*	0	0
		62.5	-.*	0	0
<i>Amaranthus hybridus</i> L.	<i>An. arabiensis</i>	2000	31.3*	20.0	44.0
		1000	3.0*	.00	16.0
		500	.7*	.00	8.0
		250	-.*	0	0
		125	-.*	0	0

		62.5	-*	0	0
	<i>An. stephensi</i>	2000	100.0*	100.0	100.0
		1000	62.3*	8.00	80.0
		500	5.0*	0	20.0
		250	.4*	0	4.0
		125	-*	0	.0
		62.5	-*	0	.0
	<i>Ae. aegypti</i>	2000	100.0*	100.0	100.0
		1000	83.3*	76.0	88.0
		500	29.3*	20.0	48.0
		250	2.0*	0	12.0
		125	-*	0	.0
		62.5	-*	0	.0
<i>Premna schimperi</i> Engl.	<i>An. arabiensis</i>	2000	100.0*	100.0	100.0
		1000	82.0*	56.0	96.0
		500	12.3*	0	32.0
		250	-*	0	0
		125	-*	0	0
		62.5	-*	0	0
	<i>Ae. aegypti</i>	2000	70.3*	56.0	80.0
		1000	56.3*	48.0	72.0
		500	1.3*	.00	12.0
		250	-*	0	0
		125	-*	0	0
		62.5	-*	0	0

Table 3. Mean percentage mortality of late 3rd - 4th instar larvae of *An.arabiensis*, *An. stephensi* and *Ae.aegypti* larvae at different concentrations of tested plant extracts after 24 h of exposure.

All values are in triplicate; -* implies no mortality

There were statistically significant differences in mean percentage mortality of the respective mosquito larvae among different concentrations of the n hexane plant extracts at ≥ 500 ppm compared with negative control ($P < 0.05$) (Table 4). Statistical results (one-way ANOVA) have showed significantly high larvicidal activity(100% mortalities) of n-hexane solvent crude extracts ($P < .000$) after 24 h exposure to four extracts of the test plants at concentrations of 2000 ppm, against *An. Arabiensis* mosquito larvae.

The present study also showed that n-hexane crude extracts of *Lepidium sativum* and *Ocimum lamiifolium* had pronounced larvicidal activity followed by *Amaranthus hybridus* and *Premna schimperi*. Significantly higher larvicidal activity ($P < 0.00$) was exerted by *Ocimum lamiifolium* at a concentration ≥ 500 ppm. Around 100% mortalities against late 3rd to early 4th instar larvae of *An.arabiensis*, *An. stephensi* and *Ae. aegypti* by *Ocimum lamiifolium* and *Amaranthus hybridus*, and *An.arabiensis* by *Lepidium*

sativum and *Premna schimperi* crude extracts were recorded respectively after 24 h of exposure at a concentration of 2000 ppm the same effect as 0.25 ppm temephos ($P > 0.05$). However, there were no statistically significant difference ($P > 0.05$) in the larvicidal potential of all n-hexane extracts at the lower concentrations ($P < 500$ ppm). The mortality effect of the test plant extracts against the test mosquitoes' larvae were dose dependent. No activity was observed in the negative control, while the positive control exhibited 100% larvae mortality (Table 4). Larvae mortality increased with increased concentrations of the tested crude extracts (Fig 1).

Table 4 indicates one-way ANOVA (statistical) results that had showed significantly higher larvicidal activity ($P < 0.00$) of the respective mosquito larvae after 24 h of exposure to *Ocimum lamiifolium*, *Lepidium sativum* and *Amaranthus hybridus* n-hexane crude extracts at concentrations ≥ 500 ppm.

Plant Species	Mosquito species	Conc. (PPM)	MD	SD	95% CI		Sig.
					LB	UB	
<i>Lepidium sativum</i> L.	<i>An. arabiensis</i>	5% DMSO	1.000	5.810	118.04	81.96	1.000
		2000	-100.000*	5.810	-118.04	-81.96	.000
		1000	-90.667*	5.810	-108.71	-72.63	.000
		500	-41.333*	5.810	-59.37	-23.29	.000
		250	-26.667*	5.810	-44.71	-8.63	.005
		125	-13.417	5.810	-31.71	4.37	.547
		62.5	-1.417	5.810	-19.71	16.37	1.000
	<i>Ae. aegypti</i>	2000	-96.750*	3.540	-113.54	-79.96	.000
		1000	-86.500*	3.540	-103.29	-69.71	.000
		500	-45.000*	3.540	-61.79	-28.21	.000
		250	-13.500	3.540	-30.29	3.29	.999
		125	-3.500	3.540	-20.29	13.29	1.000
		62.5	.000	3.540	-16.79	16.79	1.000
<i>Ocimum lamiifolium</i> Hochst. ex. Walp.	<i>An. arabiensis</i>	2000	-100.000*	4.672	-106.17	-77.16	.000
		1000	-93.00*	4.672	-99.17	-70.16	.000
		500	-17.000*	4.672	-23.17	5.84	.585
		250	-10.000*	4.672	-16.51	12.51	1.000
		125	-6.000	4.672	-12.17	16.84	1.000
		62.5	-1.583	4.672	-106.17	-77.16	.000
	<i>An. stephensi</i>	2000	-99.667*	.691	-101.81	-97.52	.000
		1000	-7.000*	.691	-9.15	-4.85	.000
		500	-0.7	.691	-2.81	1.48	.978
		250	-.333	.691	-2.48	1.81	1.000
		125	.000	.691	-2.15	2.15	1.000
		62.5	.000	.691	-2.15	2.15	1.000
	<i>Ae. aegypti</i>	2000	-100.000*	.744	-102.31	-97.69	.000
		1000	-91.750*	.744	-93.31	-88.69	.000
		500	-8.000*	.744	-10.31	-5.69	.000
		250	.000	.744	-2.31	2.31	1.000
		125	.000	.744	-2.31	2.31	1.000
		62.5	.000	.744	-2.31	2.31	1.000
<i>Amaranthus hybridus</i> L.	<i>An. arabiensis</i>	2000	-31.333*	1.298	-35.36	-27.30	.000
		1000	-3.000	1.298	-7.03	1.03	.299
		500	-.667	1.298	-4.70	3.36	1.000

	<i>An. stephensi</i>	250	.000	1.298	-4.03	4.03	1.000
		125	.000	1.298	-4.03	4.03	1.000
		62.5	.000	1.298	-4.03	4.03	1.000
		2000	-100.000*	.734	-109.11	-90.89	.000
		1000	-62.333*	.734	-71.44	-53.22	.000
		500	-5.000	.734	-14.11	4.11	.685
		250	-.333	.734	-9.44	8.78	1.000
		125	.000	.734	-9.11	9.11	1.000
	<i>Ae. aegypti</i>	62.5	.000	.734	-9.11	9.11	1.000
		2000	-100.000*	1.449	-104.50	-95.50	.000
		1000	-83.333*	1.449	-87.83	-78.83	.000
		500	-29.333*	1.449	-33.83	-24.83	.000
		250	-2.500	1.449	-6.50	2.50	.864
		125	.000	1.449	-4.50	4.50	1.000
		62.5	.000	1.449	-4.50	4.50	1.000
<i>Premna schimperi</i> Engl.	<i>An. arabiensis</i>	2000	-100.000*	1.999	-106.21	-93.79	.000
		1000	-82.500*	1.999	-88.21	-75.79	.000
		500	-12.083*	1.999	-18.54	-6.13	.000
		250	.000	1.999	-6.21	6.21	1.000
		125	.000	1.999	-6.21	6.21	1.000
		62.5	.000	1.999	-6.21	6.21	1.000
	<i>Ae. aegypti</i>	2000	-70.333	1.441	-74.81	-65.86	.000
		1000	-56.33	1.441	-60.81	-51.86	.000
		500	-1.333	1.441	-5.81	3.14	.983
		250	.000	1.441	-4.47	4.47	1.000
		125	.000	1.441	-4.47	4.47	1.000
		62.5	.000	1.441	-4.47	4.47	1.000
		Temephos 0.25	-100.000	5.810	-118.04	-81.96	.000

Table 4. Results of Post Hoc Test of Multiple Comparisons of the test plant species against larvae of *An. arabiensis*, *An. Stephensi*, and *Ae. aegypti* at different concentrations after 24 h of exposure. All values are in triplicates, *the mean difference is significant at the 0.05 level, MD-Mean Difference, SD-Std. Err.

Determination of LC₅₀ and LC₉₀ of the Crude Extracts

The lethal effects of four different test plant species crude extracts against larvae of the targeted vector mosquitos weresubjected to probit analysis. The n-hexane crude extracts of *Ocimum lamiifolium*, *Lepidium sativum*, *Amaranthus hybridus* and *Premna schimperi* plant extracts against larvae of *An. arabiensis*, *An. stephensi* and *Ae. aegypti* are shown in (Table 5).

Plant extracts	Mosquito species	χ^2	LC ₅₀	95% CI		LC ₉₀	95% CI	
				LB	UB		LB	UB
<i>Lepidium sativum</i>	<i>An. arabiensis</i> (b,c)	5.7	545.36	451.61	669.48	982.160	822.99	1260.60
	<i>Ae. Aegypti</i> (b,c)	6.22	642.24	423.37	755.90	1073.86	955.99	1260.06
<i>Ocimum lamiifolium</i>	<i>An. arabiensis</i> (b,c)	13.80	843.70	633.50	1388.21	1386.79	1016.89	2452.24
	<i>An. stephensi</i> (b,c)	19.83	1278.22	940.42	4537.28	1717.44	1309.95	9365.05
	<i>Ae. aegypti</i> (b,c)	.004	713.25	622.76	815.92	988.90	858.00	1255.86
<i>Amaranthus hybridus</i>	<i>An. arabiensis</i> (b,c)	.408	2333.96	1985.3	3246.98	3225.87	2623.69	5155.50
	<i>An. stephensi</i> (b,c)	.963	874.78	744.23	1031.01	1240.27	1092.87	1545.77
	<i>Ae. aegypti</i> (b,c)	.167	636.76	536.86	755.90	1046.01	915.37	1260.06
<i>Premna schimperi</i>	<i>An. arabiensis</i> (b,c)	.050	736.15	633.92	854.78	1073.76	955.99	1271.54
	<i>Ae. aegypti</i> (b,c)	6.38	1209.30	987.32	1525.25	2230.20	1538.14	7879.38
A) *LC₅₀- Lethal concentration that kills 50% of the exposed larvae, LC₉₀- Lethal concentration that kills 90% of the exposed larvae, χ^2- chi-square, LB- lower boundary limit, UB- Upper boundary limit B) A heterogeneity factor is used for split file Botanic crude extracts= <i>Ocimum lamiifolium</i>, exposed mosquito species = <i>An. arabiensis</i> mosquito larvae. C) A heterogeneity factor is used for split file Botanic crude extracts = <i>Ocimum lamiifolium</i>, exposed mosquito species = <i>An. stephensi</i> mosquito larvae.								

Table 5: Lethal concentration of plant crude extracts against larvae of *An. arabiensis*, *An. Stephensi* & *Ae.aegypti* after 24 h of exposure (ppm) *According to probit analysis (Finney, 1971) % Mortality= mean $\pm$ SD

Discussion

Due to environmental concerns and the development of insect resistance to synthetic insecticides, the recent trend is to evaluate plants to obtain extracts that are safe for non-target animals and do not pose any residue problem but are still able to suppress vector populations. In view of this, the present study tested the larvicidal activities of n-hexane and 80% methanol crude extracts of *Ocimum lamiifolium*, *Lepidium sativum*, *Amaranthus hybridus* and *Premna schimperi* plants against *An. arabiensis*, *An. stephensi*, and *Ae. aegypti*. In this study, all 80% methanol crude extracts showed little/no insecticidal activities on the targeted malaria and viral infections vectors, while n-hexane crude extracts of the test plants achieved lower, moderate, and high larvicidal activities. This suggests the presence of more non-polar solvent-soluble phytochemicals in the tested plant species and parts (*Ocimum lamiifolium*, *Lepidium sativum*, *Amaranthus hybridus* and *Premna schimperi*) which are responsible for the observed bioactivities of these plants against larvae of *An. arabiensis*, *An. Stephensi* and *Ae. aegypti* mosquitoes.

The rationale for the use of solvents with different polarity such as water with methanol (P= 0.762), and n-hexane (P= 0.009) and methanol gradient with water such as 80% methanol was because different organic solvents and their combination with water

show difference in dissolving the bioactive components present in the plant materials. Use of different solvents with differing polarities is necessary because different solvents can significantly affect the potency of extracted plant compounds [21]. This has also been shown by [22] in oak gall extraction and [23] in neem plant extraction, whereby a converse relationship between extract effectiveness and solvent polarity was observed. [24] Had also reported the extraction of more bioactive components from *Acorus calms* (Acoraceae) that had more lethal effect on adult mosquitoes with certain solvents than others.

This is consistent with earlier reports [25] that showed decline in mortality of mosquitoes with increasing solvent polarity of a mosquitocidal plant extract. These authors showed that water extract of *Zanthoxylum heitzii* (Rutaceae) produced low adult mortalities whereas its ethyl acetate and n-hexane extracts produced higher mortalities on *Anopheles gambiae*. From this, it is clear that the bioactive components responsible for the lethal effect on mosquitoes are extracted in greater amount and potency with certain solvents only and not with all. The current finding of bioassay of polar (80% methanol) and non-polar (n-hexane) plant extracts against *An. arabiensis*, *An. stephensi* and *Ae. aegypti* larvae was in line with findings of earlier studies. The present study demonstrated that the larvicidal activity of n-hexane extracts were much higher compared to the 80% methanol extracts implies that the potency of the active constituents in the crude extracts might have been masked by non-polar and major/ minor constituents.

All the n-hexane extracts showed larvicidal activities against larvae of *An. arabiensis* and *Ae. aegypti* mosquitos. Among the tested extracts, the high larvicidal activity shown by n-hexane extract of *Ocimum lamiifolium* against the larvae of the three mosquito vectors in Ethiopia is not surprising since it has been reported as a multipurpose traditional medicine. Due to its pharmacological effects, this plant has been widely used traditionally for the treatment of headaches, coughs, diarrhea, constipation, warts, and kidney damage [26]. These properties come from the secondary metabolite components that are abundant in *Ocimum* plants such as steroids, tannins, alkaloids, flavonoids, and phenolics [27]. In addition, the abundant components of essential oils make *Ocimum* a plant that can fight the growth of organisms [28]. *Ocimum lamiifolium* has many pharmacological properties that is the reason why they are well-known, praised, and widely used as home remedies [29].

The most widely used mosquito repellent plant reported in Ethiopia is *Allium sativum* L. followed by *Lepidium sativum* L. and *Capparis tomentosa* Lam. [30, 31]. [31] Also reported that insecticides were made locally to kill insects by spraying all over the walls of the house. It is plausible to assume that it is the phytochemicals contained in such plants that are responsible for the insecticidal activities against both the larvae and adult stages of mosquitoes.

The findings of the present study also indicated that n-hexane crude extract of *Amaranthus hybridus* showed statistically significant larvicidal activity ($P < 0.000$) against late 3rd - early 4th instars larvae of *An. arabiensis*, *An. stephensi* and *Ae. aegypti* at concentrations of 2000, ≥ 1000 , and ≥ 500 ppm respectively after 24 h exposure. The result of this finding is consistent with the research, [32] which showed the larvicidal activity of the same plant extract and their Cu nanoparticles (extract materials sized, from 1 to 100 nanometers in diameter) against *Culex* and *anopheles*' larvae. The highest mortality was found at 50 mg/L with the lethal dose or lethal concentration (LC) of 50% and 90% mortality calculated to be 0.861 and 0.995 respectively. The mortality is attributed to the presence of phytochemicals in the leaves of the plant extract. The current work also can be compared to the work of [33]. Similarly, the finding of [34] where ethanol leaf extract of *D. stramonium* was found to cause 70.56% mortality against 3rd instar *An. gambiae* larvae at 1000 ppm in Eritrea though the concentrations are different. The petroleum ether extract of *Datura stramonium* also showed strong efficacy against the 4th instar larvae *Aedes aegypti* 100% mortality after 24 h of exposure [35]. The difference in larvicidal activity in the current finding could be due to the difference in species and parts of plants, concentrations, extraction solvent, and season of collection and agro ecology of the plants.

The biological activities of the phytochemicals that include alkaloids, terpenoids, steroids, phenols, saponins and tannins extracted from several tropical plants have been receiving the attention of many researchers as potential sources of mosquitocides and for treatment of various vector-borne diseases [21, 36]. The crude extracts that caused high mortality to mosquitos' larvae contained plant secondary metabolites with such as the alkaloids, phenols, flavonoids, saponins, cardiac glycosides, detected are among substances with insecticidal effects [32, 37]. Therefore, the high larvicidal potencies of n-hexane crude extracts of the four plants were the basis for considering further screening studies on the different solvent partitioned fractions against the three targeted mosquito vectors.

The ethyl acetate extract of *L. sativum* damaged the midgut of *Cx. pipiens* larvae, interfering with development and survival. A similar result was obtained when *Aedes aegypti* (Linnaeus in Hasselquist) (Diptera: Culicidae) larvae were treated with *Schinus terebinthifolius* Raddi (Sapindales: Anacardiaceae) extract, resulting in disorganization and damage in the midgut in comparison to the control [38]. Different plant derived larvicides caused deleterious effects in the larvae midgut, including vacuolization, cell hypertrophy, damage to microvilli, cell lysis, degeneration of epithelial cells, disruption of osmoregulation, and damage to the gastric caeca [39,40,41]. The remarkable larvicidal and ovicidal activities of *L. sativum* ethyl acetate extract might be due to the presence of phenol, which is known to possess promising insecticidal activity. Several compounds from different plants such as phenolics, steroids, alkaloids, essential oils, and terpenoids have been reported as promising potential insecticides [21], either in pure or crude extract form. The larvicidal effects of plant secondary metabolites vary based on plant species, parts used, geographical region, mosquito species, extraction methodology, and the polarity of the solvents used during extraction [42]. Due to the fact, all 80% methanol crude extracts caused lower larvicidal activities, only n-hexane solvents crude extracts were subjected to dose response bioassay to detect the lethal concentrations. The n-hexane extract of *Lepidium sativum* caused the highest larvicidal activities against the late 3rd to early 4th instar larvae of *An. arabiensis* and *Ae. aegypti* with the lowest LC₅₀ of 545.360 ppm and LC₉₀ of 982.160, *Amaranthus hybridus* showed the relatively highest LC₅₀ and LC₉₀ (LC₅₀ = 2857.24 ppm, LC₉₀ = 6683.15 ppm) and, 642.244 and 1073.864 respectively. Similarly, the highest larvicidal activities against *An. stephensi* larvae were exhibited by n-hexane extract of *Amaranthus hybridus* with the lowest LC₅₀ of 874.78 ppm and LC₉₀ of 1240.27 ppm values. This difference could be arisen due to difference in environmental factors. In his review, [43] indicated that plant secondary metabolite accumulation is strongly dependent on a variety of environmental factors such as light, temperature, soil water, soil fertility and salinity, and for most plants, a change in an individual factor may alter the content of secondary metabolites even if other factors remain constant.

The highest larvicidal activities against *An. stephensi* larvae were exhibited by n-hexane extract of *Amaranthus hybridus* with the lowest LC₅₀ of 874.78 ppm and LC₉₀ of 1240.27 ppm values. Similarly, [44] reported that, petroleum ether extract of leaf powder *Amaranthus hybridus* resulted in the LC₅₀ of 409.87 ppm against 3rd instar larvae of *Culex* species after 24 h of exposure. The difference in larvicidal activity might be occurred due to difference in susceptibility of the test species and solvent type. The difference in larvicidal activity in the current finding might be due to the above-described reasons. Therefore, we recommend bioassay guided fractionation, isolation, and characterization of the responsible active constituents of the four plants crude extracts.

Conclusion

This study showed that the n-hexane crude extracts of *Ocimum lamiifolium*, *Lepidium sativum*, and *Premna schimperi* could be considered as foreseeable products to be developed as potential larvicides against *Anopheles arabiensis* and *Aedes aegypti* mosquito larvae. In addition, n-hexane crude extract of *Ocimum lamiifolium* had potent larvicidal activity against *An. stephensi* larvae. The 100% larvae mortality obtained with 2000 ppm crude extract of the test plant *Amaranthus hybridus* against the *An. stephensi* and *Aedes aegypti* mosquito larvae is an indication of a very good potential that the four test plant species that can be easily cultivated, could be used to extract products for use as larvae control agents against the respective three targeted mosquito vectors of malaria and viral infections namely *Anopheles arabiensis*, *Anopheles stephensi* and *Aedes aegypti*. There is, however, a need for further investigation on their efficacy under natural conditions so that can be potential larvicides in the fight against malaria vectors in endemic areas. These four plants could be used to develop effective, safe, biodegradable, and cheap botanical insecticides for vector control, potentially leading to improved resistance management targeted against respective larvae of mosquito vectors of malaria and viral infection in Ethiopia and elsewhere. Therefore, further chemical analysis studies on the identification, preparation, and formulation of bioactive compounds from these tested plants are recommended.

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

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Authors' Contributions

N.G. designed the research, conducted data collection, analysis, and wrote the initial & final draft of the manuscript. S.D. conceived the idea, designed the research, acquired funding and involved in the write up. K.T. designed the research, funding acquisition, supervised the data collection, confirmed plant identification and involved in the write up of the manuscript. A.G. involved in the designing of the research, contributed to the write up and reviewed the manuscript. E.A. involved in the designing of the research, and reviewed the manuscript. D.B. and B.K. involved in the planning and execution of the research. All authors read and approved the final manuscript.

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Availability of Data and Materials

The datasets used and/or analyzed during the current study may be obtained from the corresponding author on reasonable request.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethics approval and consent to participate

The field data collection was conducted after receiving ethical clearance certificate from Institutional Research Ethics Review Committee (ALIPB-IRERC) at Aklilu Lemma Institute of Health Research, Addis Ababa University, with reference number: ALIP-IRERC/140/2024).

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Figures

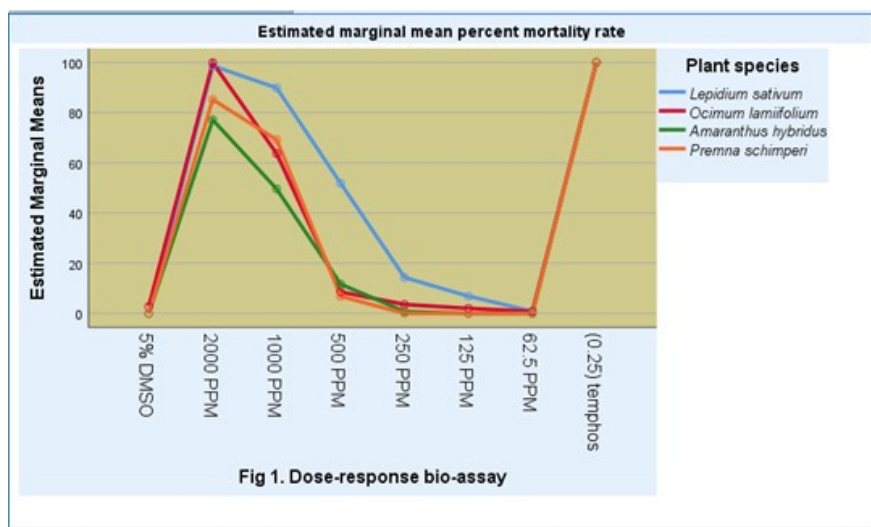


Figure 1

Indicated the larvicidal activity of different concentrations of n-hexane crude extracts of *Ocimum lamiifolium*, *Lepidium sativum*, *Amaranthus hybridus* and *Premna schimperi* plants against *An. arabiensis*, *An. stephensi*, and *Ae.aegypti.stephensi* and *Ae.aegypti*.