

Larvicidal, Ovicidal and Repellent Activities of *Leucas stachydiformis* (Hochst. ex Benth.) Briq Essential Oil against *Anopheles arabiensis*

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Abstract

Background: Despite tremendous efforts made to curb malaria morbidity and mortality, malaria control has slowed down due to resistance to all currently used insecticides by mosquitoes, and their negative effect on the non-target organisms and the ecosystem. Thus, there is an urgent need to search and develop new plant-based malaria vector control strategy from medicinal plants origin such as essential oil.

Methods: Larvicidal effect was tested against the fourth instar *An. arabiensis* wild larvae whilst freshly laid ova of *An. arabiensis* were used to determine ovicidal activity of the essential oil at concentrations ranging from 6.25 to 400 ppm. Concentrations of 41.6–366.7 $\mu\text{g}/\text{cm}^2$ were used to evaluate repellent activity of the essential oil on 3–5 days old adult female *An. Arabiensis*. The essential oil composition of *Leucas stachydiformis* was also analyzed using GC-MS.

Results: Larvicidal, ovicidal and repellent activities of the essential oil extracted by hydrodistillation from leaves of the endemic Ethiopian plant *Leucas stachydiformis* (Hochst. ex Benth.) Briq was investigated against *Anopheles arabiensis*, the dominant malaria vector species in Ethiopia. The study revealed that the oil possesses the highest larvicidal activity at 400 ppm and 200 ppm after 24 h and 48 h treatment. LC_{50} values for the fourth larval instar after 24 h and 48 h treatment were 43.4 ppm and 34.2 ppm, respectively. After 72 h exposure, the oil displayed 100% ovicidal activity at 400 ppm with IH_{50} value of 32.2 ppm. In the repellency test, at concentrations of 366.7, 133.3 and 41.6 $\mu\text{g}/\text{cm}^2$, the oil gave total percentage protection of $67.9 \pm 4.2\%$, $37.2 \pm 2.8\%$ and $32 \pm 2.2\%$, respectively, for 4 hours. The highest concentration (366.7 $\mu\text{g}/\text{cm}^2$) gave 100% protection up to 90 min. GC-MS analyses of the oil revealed the presence of 24 compounds representing 90.34% of the total oil with caryophyllene oxide, germacrene D, and *trans*-caryophyllene constituting more than 50% of its components.

Conclusion: Results of the present study suggest that the essential oil of *L. stachydiformis* has the potential to be used for the control of *An. arabiensis* mosquitos.

Background

Despite tremendous efforts made to curb malaria morbidity and mortality, further progress in malaria control has slowed down recently [1]. According to the latest WHO report, globally, there were 247 million cases of malaria in 2021 compared to 245 million cases in 2020 [2]. Vector control is one of the prevention tools to mitigate mosquito-borne diseases including malaria [3]. In this regard, community based long-lasting insecticidal nets (LLINs), and larval source management (LSM) have been the mainstay in vector control efforts [4]. However, these synthetic insecticide-based methods are threatened because of widespread resistance to all currently used insecticides by mosquitoes, and their negative effect on the non-target organisms and the ecosystem [5]. These problems may lead to renewed outbreaks of malaria as well as deaths due to insecticidal poisoning. Above all, the situation is worsening

in developing countries as the control of malaria vectors predominantly relies on these insecticide-based methods and also due to improper use of personal protections [6, 7].

Thus, there is an urgent need to search and develop new malaria vector control methods which are more environmentally safe, biodegradable and target-specific insecticides [8]. Identification of potential insecticides and repellents from natural product resources, such as plant extract and essential oils is one solution for the above problems. Previous studies have demonstrated that members of the genus *Leucas* possess larvicidal [9–12], ovicidal [13] and repellent [14] properties against different stages in the life cycle of mosquito vectors. Therefore, the present study was designed to investigate the larvicidal, ovicidal, and repellent activities of the essential oil of leaves of *Leucas stachydiformis* (Hochst. ex Benth.) Briq against *An. Arabiensis*, the dominant malaria vector in Ethiopia.

Methods

Plant material and extraction of essential oil

The leaves of *L. stachydiformis* were collected from Gulelle Botanical Garden (GBG), Addis Ababa, Ethiopia. The authenticity of the plant material was confirmed by Dr. Birhanu Belay of GBG and a voucher specimen with collection number SF001/2021 was deposited at the National Herbarium, Department of Biology, Addis Ababa University, Ethiopia. The essential oil was extracted by hydrodistillation using Clevenger-type apparatus. The residual water from the oil was removed by anhydrous sodium sulfate and the oil was stored in a refrigerator in a tightly closed amber colored bottle until used for GC-MS analysis and bioassays.

Gas chromatography-mass spectrometry (GC-MS) analysis

GC-MS analysis was carried out on 7890A GC System (Agilent Technologies, Greece) fitted with a non-polar column; HP-5 MS, capillary column size of 30 m x 0.25 mm I.D and 0.25 µm film thickness. Prior to analysis, the essential oil was diluted with *n*-hexane in 10:1 ratio. The diluted essential oil (1 µl) was injected at 240°C by using helium as carrier gas at flow rate of 0.9 ml/min. Then the gas chromatograph oven temperature was programmed to 60°C for 1 min, then set at rate of 3°C/min until the temperature reached 240°C. The interface temperature was 280°C. When the GC separated component of the essential oil reached the mass spectrometer (5975C VL MSD, Agilent Technologies, Greece), it was ionized by 70 eV ionization voltage with source temperature of 230°C. The ionized samples were accelerated and separated according to mass to charge ratio (*m/z*) by quadrupole mass analyzer with a temperature of 150°C. Finally, the ionized species were detected by MS detector with scanning capacity of 40–400 amu. The compounds were identified by comparing the obtained mass spectra with the mass spectra from the database.

Larvicidal bioassay

The larvicidal bioassay test of extracts and essential oil was carried out following the standard WHO protocol [15]. A group of fourth instar *An. arabiensis* larvae (*n* = 25) were immersed by using dropper to

white enamel cups that contain water and various concentrations of test samples (6.25, 12.5, 25, 50, 100, 200, and 400 ppm). Then, the cups were kept at a temperature of $27 \pm 2^{\circ}\text{C}$ and relative humidity of $75 \pm 10\%$ with a photoperiod of 12 h light and 12 h dark cycles. The experiment was conducted in four replicates for each concentration of test samples and controls. Larval mortalities were recorded after 24 and 48 h of exposure and then, percentage of larval mortalities was calculated using the formula shown in the equation below

$$\text{Percentage of larval mortality} = \frac{\text{Number of dead larvae}}{\text{Total larvae introduced}} \times 100$$

Ovicidal activity test

The test substances were assayed for their ovicidal activity against freshly laid *An. arabiensis* eggs as per the method described by Elango *et al.* [16] with minor modifications. The same test concentrations were used as in larvicidal bioassay tests. *An. arabiensis* eggs ($n = 25$) were immersed by using a dropper to white enamel cups that contain different concentrations of the oil. Egg hatchability was assessed after incubation for 72 h. Ovicidal effect was calculated in percentage by dividing the number of hatched eggs by total number of eggs immersed times 100 as shown in equation below.

$$\text{percentage hatchability} = \frac{\text{Number of hatched eggs}}{\text{Total number of eggs}} \times 100$$

Repellent activity test

Repellency activity of the test substances against adult female *An. arabiensis* was performed following the WHO mosquito repellence test protocol [17]. Fifty nulliparous 3–5 days old female mosquitos starved for about 12 h were placed into a test cage during all tests in the laboratory. Before the commencement of the experiment, three volunteers washed both their hands and forearms with scent free soaps and dry them for 10 min before extracts and control application.

One ml each of 366.7, 133.3 and 41.6 $\mu\text{g}/\text{cm}^2$ of the essential oil and ethanol (diluent) was applied to approximately 600 cm^2 of forearm. The treated and control arms were placed every 30 min in the mosquito cage for 3 min observation period. The period of observation was 30, 60, 90, 120, 150, 180, 210, and 240 min. Mosquitoes that land/probe on the forearm were recorded. Tests were conducted during the day in the dark room. Each test concentration was repeated three times with three volunteers. Formulated commercial 20% N, N-diethyl-meta-toluamide (DEET) was used as a standard for comparison of protection time to that of the test repellents. Percentage of protection is calculated for each concentration by using the formula below

$$\text{Percentage of protection} = \frac{C-T}{C} \times 100$$

Where C and T are the number of mosquito landings on the forearm of negative control and treated skin, respectively.

Results and Discussion

Chemical composition

Hydrodistillation of the dried leaves of *L. stachydiformis* yielded 0.36% (w/w) a pale yellow-colored essential oil with distinct smell. GC-MS analysis identified a total of 24 compounds representing 90.34% of the total oil. The essential oil was found to be rich in sesquiterpene hydrocarbons (45.07%), followed by oxygenated sesquiterpenes (30.27%), oxygenated monoterpenes (7.03%), diterpene hydrocarbons (6.81%), monoterpene hydrocarbons (0.24%) and others (0.92%). As shown in Table 1, the major components of the oil were caryophyllene oxide (19.84%), germacrene D (19.06%), *trans*-caryophyllene (12.29%), phytol (6.81%), α -humulene (4.81%), humulene epoxide II (3.57%), linalool (3.33%), and spathulenol (2.77%). Although the major components of the oil were previously isolated from other *Leucas* species, this is the first report on the identification of the monoterpenes, *trans*-verbenol (1.97%) and verbenone (0.46%) and the sesquiterpene hydrocarbon modheph-2-ene (0.37%) in the genus *Leucas*.

Larvicidal activity

As shown in Table 2, the essential oil of *L. stachydiformis* exerted concentration dependent larvicidal activity against fourth instar *An. arabis* larvae. At a concentration of 400 ppm, 92% of the larvae were killed within 24 h of exposure then rose to 96% after 48 h of exposure (Table 2). According to Oliveros *et al.* [18] larvicidal efficiency classification, the essential oil of *L. stachydiformis* showed high larvicidal activity at a concentration of 400 ppm and 200 ppm without significant difference after 24 h and 48 h exposure ($p > 0.05$). However, at concentrations less than 200 ppm, the activity was concentration dependent ($p < 0.05$). This result agrees with the findings of Thomas *et al.* [19], where a concentration dependent larvicidal action was observed for the essential oils of *Syzygium aromaticum* (clove) and *Cinnamomum verum* (cinnamon) against *An. gambiae*. The mortality rate observed for the negative control after 24 and 48 h exposure was 0%, and all the larvae were developed into pupae after 48 h.

After 48 h exposure, the LC_{50} and LC_{90} values of *L. stachydiformis* essential oil were 34.2 ppm and 175.7 ppm, respectively (Table 2).

Table 1

Chemical compositions of the essential oil isolated from the leaves of *Leucas stachydiformis*

No.	Components	RT ^a	RI(HP5-MS) ^b	Area or content (%)
1	α -Pinene	5.19	931	0.24
2	1-Octen-3-ol	6.37	977	0.92
3	Linalool	10.48	1100	3.33
4	<i>trans</i> -Verbenol	12.21	1142	1.97
5	α -Terpineol	14.10	1189	1.26
6	Verbenone	14.84	1207	0.46
7	Modheph-2-ene	21.93	1376	0.37
8	β -Bourbonene	22.18	1382	1.49
9	<i>trans</i> -Caryophyllene	23.57	1415	12.29
10	α -Humulene	24.94	1450	4.81
11	γ -Muurolene	25.92	1474	2.00
12	Germacrene D	26.07	1478	19.06
13	<i>trans</i> - β -Ionone	26.31	1483	0.55
14	Bicyclogermacrene	26.68	1493	1.62
15	δ -Cadinene	27.78	1521	0.54
16	Germacrene B	28.99	1552	2.35
17	Spathulenol	29.79	1573	2.77
18	Caryophyllene oxide	29.98	1578	19.84
19	Humulene epoxide II	30.96	1604	3.57
20	τ -Cadinol	32.20	1638	1.14
21	α -Cadinol	32.69	1651	1.55
22	15-Hydroxy- α -muurolene	33.81	1682	0.38
23	6,10,14-Trimethyl-2-pentadecanone	39.47	1844	1.04
24	Phytol	47.90	2111	6.81
Sesquiterpene hydrocarbons				45.07%
Oxygenated sesquiterpenes				30.27%

No.	Components	RT ^a	RI(HP5-MS) ^b	Area or content (%)
	Oxygenated monoterpene			7.03%
	Hydrogenated diterpene			6.81%
	Monoterpene hydrocarbons			0.24%
	Others			0.92%
	Total identified			90.34%

^aRT is retention time, ^bRI is Kovats retention indices determined relative to a series of *n*-alkanes (C9–C29) on a non-polar (HP5-MS capillary) column.

Table 2

Larvicidal activity of essential oil from *Leucas stachydiformis* against fourth instar *Anopheles arabiensis* larvae

Concentration (ppm)	Mortality % ± SD			LC ₅₀ (95% CI) ppm	LC ₉₀ (95% CI) ppm	Chi-square
	24 h	48 h	24 h			
400	92 ± 0.5	96 ± 0.7		43.4	229.7	6.3
200	88 ± 1.2	92 ± 1.5		(31.8–58.5)	(151.6–430.9)	
100	72 ± 0.9	72 ± 1.3				
50	56 ± 1.3	68 ± 1.1				
25	52 ± 2.5	60 ± 1.8	48 h	34.2	175.7	8.3
12.5	8 ± 0.6	8 ± 1.6		(25.0–45.7)	(118.3–317.9)	
6.25	4 ± 0.2	8 ± 0.5				
Negative control	0 ± 0.0	0 ± 0.0				

Values were based on seven concentrations and four replications with 25 larvae in each; Chi-square values were significant at $p < 0.05$ level; LC₅₀: 50% lethal concentration; LC₉₀: 90% lethal concentration; CI: confidence interval; Negative control: 2% dimethyl sulfoxide (DMSO).

The 24 h LC₅₀ value of the essential oil of *L. stachydiformis* (43.4 ppm) was very similar to that of *L. zeylanica* essential oil (44 ppm) against *Ae. Aegypti*. This similarity in potency could be due to the

presence of high amounts of caryophyllene oxide, germacrene D, phytol and α -humulene in both essential oils [20].

Larvicidal properties of the essential oil of *L. stachydiformis* may be linked to its major constituents such as caryophyllene oxide, germacrene D, linalool, α -humulene which have been reported to have strong larvicidal properties [20–23]. But, the effect of these major constituents may have been strengthened by the co-occurrence of minor compounds that act in an additive and/or synergistic manner [24]. Among the minor constituents of the oil, α -pinene, verbenone, bicyclogermacrene, δ -cadinene, and α -terpineol were reported to have larvicidal activity [1, 22, 25, 26]

Different phytochemicals are known to cause neurotoxicity or direct contact toxicity to the midgut epithelium of mosquito larvae [27]. In the present study, the identified essential oil constituents such as caryophyllene oxide, α -pinene, linalool are known to have anti-acetylcholinesterase activity [28], whereas α -terpineol was reported to have influence on the production of cyclic adenosine monophosphate (cAMP) and calcium at the cellular level and these effects consequentially lead to larval death [29].

It was reported that the presence of a variety of compounds in essential oils may lead to a collaborative cell membrane penetration of the mosquito larvae by non-polar constituents thereby enhancing the entry of active compounds to the cell, and this effect consequently increases their bioavailability and larvicidal activity [30]. This combination (synergistic) effect may have occurred in the essential oil of *L. stachydiformis* since the main constituents identified are highly lipophilic compounds. It has been reported that combinatorial action does not always lead to positive effect, i.e. the isolated pure compounds may have greater larvicidal effect than the essential oil from which the compound is isolated. For example, Govindarajan *et al.* [31] reported that the larvicidal activity of pure (Z)- γ -bisabolene isolated from the essential oil of *Galinsoga parviflora* (Asteraceae) is much higher than that of the oil itself.

Ovicidal activity

Controlling of egg hatching before larval emergence is another efficient approach for malarial vector management [32]. In the present study, the mean percentage hatchability of the eggs was observed post 72 h. It was found that the mean percentage hatchability was inversely proportional to the concentration of the essential oil. The essential oil of *L. stachydiformis* showed mean percentage hatching that ranges from 0% at 400 ppm to 90% at 6.25 ppm (Table 3).

Table 3
Hatchability of *Anopheles arabiensis* eggs after 72 h exposure with essential oil of *Leucas stachydiformis*

Concentration (ppm)	Hatchability % $\pm$ SD	IH ₅₀ (95% CI) ppm	IH ₉₀ (95% CI) ppm	Chi-square
400	0 $\pm$ 0.0	32.2	144.7	4.1
200	16 $\pm$ 2.2	(24.1–42.5)	(99.8–252.3)	
100	25 $\pm$ 3.5			
50	36 $\pm$ 1.4			
25	50 $\pm$ 1.2			
12.5	85 $\pm$ 2.5			
6.25	90 $\pm$ 1.5			
Negative control	100 $\pm$ 0.0			

Values were based on seven concentrations and four replications with 25 eggs in each test cup. Chi square value are significant at $p < 0.05$ level. IH₅₀: 50% hatching inhibition concentration; IH₉₀: 90% hatching inhibition concentration; CI: confidence interval.

The data from probit analysis revealed that more concentrated oil was needed to reach 50% hatching inhibition than killing 50% of the larvae. This could be due to the fact that eggs are encapsulated by chorionic membrane which make them more resistant to physical or chemical stress [33].

Repellent activity

Repellents as a method of preventing malaria are particularly promising in regions where vector mosquitoes bite early in the evening and have exophagic (outdoor) feeding habits. In such cases, bed net use is unlikely to be very effective [34]. As shown in Fig. 1, the mean percentage of repellency (MPR) of the essential oil at different concentrations in ethanol was compared with 20% DEET in 30 min interval until 240 min. The oil gave protection against mosquito bites without any allergic reaction to the test persons. The total percentage protection of the oil for 4 h was $67.9 \pm 4.2\%$ at $366.7 \mu\text{g}/\text{cm}^2$, $37.2 \pm 2.8\%$ at $133.3 \mu\text{g}/\text{cm}^2$ and $32 \pm 2.2\%$ at $41.6 \mu\text{g}/\text{cm}^2$. The $96.7 \pm 2.2\%$ MPR of 8% ($133.3 \mu\text{g}/\text{cm}^2$) of the oil in 30 min was almost equivalent to 1000 ppm (0.1%) of the essential oil of *Millettia ferruginea* which gave $97.12 \pm 1.67\%$ protection against *An. arabiensis* in 20 min [35].

Among the tested concentrations, the highest MPR was recorded for the 22% ($366.7 \mu\text{g}/\text{cm}^2$) oil which provided repellency percentage of 100% in the first 90 min [complete protection time (CPR)], but gradually decreased to $67.9 \pm 4.2\%$ after 240 min (4 h). The 4 h MPR of this concentration was comparable to 20% *Cymbopogon nardus* that generated protection of around 70% at 4 h observation against *An. Arabiensis* [36].

According to Dugassa *et al.* [37], *An. arabiensis* have peaks occurring in both the first and second half of the night. Therefore, the essential oil of *L. stachydiformis* may not be suitable to use for the whole night because of its short CPT (90 min). However, it can be applied in the early evening i.e. before bed time to reduce the early biting of mosquitoes, and the late indoor biting can be prevented by mosquito nets.

Repellent properties of the essential oil of *L. stachydiformis* may be associated with the presence of monoterpenoids and sesquiterpenes [38]. The major constituents of the oil i.e. caryophyllene oxide and germacrene D have been reported to have strong repellent activities [33, 39]. According to Shoukat *et al.* [40], these compounds are less volatile and have a long-lasting effect as repellent. Similarly, among the minor constituents of the oil, α -terpineol [41], linalool [42], α -pinene [43], and δ -cadinene [44] have demonstrated mosquito repellent effect. Therefore, the mosquito repellent effect of *L. stachydiformis* oil against *An. arabiensis* is in full or in part attributed to the above-mentioned compounds.

Generally, phytochemicals do also have an advantage since their toxic effects to non-target organisms is less, and they are biodegradable and disappear from the environment within a short period of time [24], whereas synthetic larvicides can continuously contaminate the water environment and toxic to non-target organisms [45].

Conclusion

The present study revealed that the essential oil that extracted by hydrodistillation of the leaves of *L. stachydiformis* was rich in sesquiterpenes hydrocarbons and oxygenated sesquiterpenes such as caryophyllene oxide, germacrene D, *trans*-caryophyllene, linalool and, phytol. Also, the essential oil demonstrated potent larvicidal, ovicidal and repellent activities against *An. arabiensis*. Thus, the current study provided evidence that the leaves of *L. stachydiformis* have the potential for development of natural insecticides for the control of mosquitoes.

Declarations

Ethical clearance

As far as the Ethical clearance concerned, the manuscript is emanated from Sisay Fikru's MSc thesis work where ethical clearance and approval secured for "Laboratory evaluation of insecticidal and repellent activity of *Lucas stachydiformis* against *Anopheles arabiensis*. Larvicidal, Ovicidal and Repellent Activities of *Leucas stachydiformis* (Hochst. ex Benth.) Briq Essential Oil against *Anopheles arabiensis* emanated from this MSc thesis research work (see the scanned Ethical clearance document).

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Availability of data and material: Any data and material will be made available upon request.

References

1. Milugo TK, Tchouassi DP, Kavishe R A, Dinglasan RR, Torto B. Naturally occurring compounds with larvicidal activity against malaria mosquitoes. *Front. in Tropical Diseases*. 2021; 2: 718804.
2. WHO. World Health Organization. Guidelines for Malaria. Geneva: World Health Organisation. 2022; accessed on 18 February 2022.
3. Eukubay A, Getu E, Debebe E, Hadis M. Larvicidal potential of some plant extracts against *Anopheles arabiensis* patton (Diptera: Culicidae). *Int. J of Tropical Insect Science*. 2021; 41: 479–485.
4. Perugini E, Guglielmo F, Gabrieli E, Ranson H. The interplay between malaria vectors and human activity accounts for high residual malaria transmission in a Burkina Faso village with universal ITN coverage. *Parasites and Vectors*. 2023; 16: 101.
5. Rants'o TA, Koekemoer LL, Panayides JL, Van RL. Potential of essential oil-based anticholinesterase insecticides against *Anopheles* vectors: A Review. *Molecules*. 2022; 27: 2481–2484.
6. Mulatier M, Cohuet A, Carrasco D. Chapter 34: Repellents for mosquito-borne disease control: beyond the repellency effect. In: *Sensory Ecology of Disease Vectors*, R Ignell, CR Lazzari, MG. Lorenzo and SR Hill (eds), Wageningen Academic Publishers, Netherlands. 2022: 879–911.
7. Moyes CL, Athinya DK, Seethaler T, Battle KE, Sinka M, Hadi MP, Hemingway J, Coleman M, Hancock PA. Evaluating insecticide resistance across african districts to aid malaria control decisions. *Proceedings of the National Academy of Sciences*, 2020; 117: 2242–2250.
8. Degu S, Berihun A, Muluye R, Gemedà H, Debebe E, Amano A, Abebe A, Woldkidan S, Tadele A. Medicinal plants that used as repellent, insecticide and larvicide in Ethiopia. *Pharmacy and Pharmacology International Journal*, 2020;8: 274–283.
9. Irrusappan H, Gokulakrishnan J, Elumalai K, Senthilmurugan S. Entomotoxicity properties of eco-friendly methanol extract fractions from *Phyllanthus nodiflorus* (L) green leaf exhibits mosquito larvicidal, pupicidal and antimicrobial activity. *World News of Nat Sci*, 2022; 42:107–122.
10. Rajan K, Archana A, Anusuya S. Larvicidal potential of certain plant extracts of the family Lamiaceae against *Anopheles subpictus* (Diptera: Culicidae). *Int J of Mosquito Research*, 2021;8: 35–42.
11. Elumalai D, Hemavathi M, Hemalatha P, Deepaa CV, Kaleena PK. Larvicidal activity of catechin isolated from *Leucas aspera* against *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus* (Diptera: Culicidae). *Parasitology Research*, 2016;115:1203–1212.
12. Théophile B, Pierre S, Dieudonné N. Evaluation of the larvicidal and pupicidal potential on *Anopheles gambiae* of methanolic extracts of *Cyperus rotundus* (Cyperaceae) and *Leucas martinicensis* (Lamiaceae). *Int J of Mosquito Research*, 2020;7: 1–7.

13. Arivoli S, Tennyson S. Effects of *Leucas aspera* (Willd.) Spreng (Lamiaceae) leaf extracts against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* (Diptera: Culicidae). World Applied Sci J, 2011;14: 565–568.
14. Tjitraresmi A, Moektiwardoyo M, Susilawati Y, Shiono Y. Antimalarial activity of Lamiaceae family plants:A review. Syst Rev in Pharmacy, 2020;11:324–333.
15. WHO. World Health Organization. Guidelines for Laboratory and Field Testing of Mosquito Larvicides. Geneva, Switzerland: World Health Organization; 2005.
16. Elango G, Bagavan A, Kamaraj C, Abdur Zahir A, Abdul Rahuman A. Oviposition-deterrent, ovicidal, and repellent activities of indigenous plant extracts against *Anopheles subpictus* Grassi (Diptera: Culicidae). Parasitology Research, 2009;105:1567–176.
17. WHO. World Health Organization. Guidelines for Efficacy testing of Mosquito Repellents for Human Skin; 2009.
18. Oliveros-díaz AF, Pájaro-González Y, Cabrera-Barraza J, Hill C, Quiñones-fletcher W, Olivero-verbel J, Fredyc C, Felipe Oliveros-Díaz A, Pájaro-González Y, Cabrera-Barraza J, Hill C, Quiñones-fletcher W, Olivero-verbel J, Díaz Castillo F. Larvicidal activity of plant extracts from Colombian north coast against *Aedes aegypti* L. mosquito larvae. Arabian J of Chem, 2022; 15: 104365.
19. Thomas A, Mazigo, HD, Manjurano A. *et al.* Evaluation of active ingredients and larvicidal activity of clove and cinnamon essential oils against *Anopheles gambiae* (*sensu lato*). Parasites Vectors, 2017;10: 411. <https://doi.org/10.1186/s13071-017-2355-6>.
20. Hung NH, Chuong NTH, Satyal P, Hieu HV, Dai DN, Huong LT, Sinh LH, Ngoc NTB, Hien VT, Setzer WN. Mosquito larvicidal activities and chemical compositions of the essential oils of *Leucas zeylanica* growing wild in Vietnam.Nat Prod Commun, 2019;14: 1934578X19842675.
21. Chan CA, Ho LY, Sit NW. Larvicidal activity and phytochemical profiling of sweet basil (*Ocimum basilicum* L.) leaf extract against Asian Tiger mosquito (*Aedes albopictus*). Horticulturae, 2022; 8: 443.
22. Hoi TM, Satyal P, Huong LT, Hau, DV, Binh TD, Duyen DTH, Dai DN, Huy NG, Chinh H, Van H, Hung NH, Setzer WN. Essential oils from vietnamese Asteraceae for environmentally friendly control of *Aedes* mosquitoes. Molecules, 2022; 27: 7961.
23. Liu XC, Liu QY, Zhou L, Liu QR, Liu ZL. Chemical composition of *Zanthoxylum avicennae* essential oil and its larvicidal activity on *Aedes albopictus* Skuse. Tropical J Pharm Res, 2014; 13: 399–404.
24. Oliveira BMS, Mel CR, Santos ACC, Nascimento LFA, Nízio DAC, Cristaldo PF, Blank AF, Bacci L. Essential oils from *Varronia curassavica* (Cordiaceae) accessions and their compounds (E)-caryophyllene and α -humulene as an alternative to control *Dorymyrmex thoracicus* (Formicidae: Dolichoderinae). Environ Sci and Pollution Res, 2019; 26: 6602–6612.
25. Govindarajan M, Benelli G. Eco-friendly larvicides from Indian plants: effectiveness of lavandulyl acetate and bicyclogermacrene on malaria, dengue and Japanese encephalitis mosquito vectors *Marimuthu Govindarajan*. Ecotox and Environ Safety, 2016;133: 395–402.

26. Daniela M, Navarro AF, Patrícia CB, Marcelo, FR, Napoleão TH. Larvicidal activity of plant and algae extracts, essential oils and isolated chemical constituents against *Aedes aegypti*. The Nat Prod J, 2013;3: 269.
27. Ganesan P, Samuel R, Mutheeswaran S, Pandikumar P, Reagan AD, Aremu AO, Ignacimuthu S. Phytocompounds for mosquito larvicidal activity and their modes of action: A review. South Afr J of Botany, 2023;152:19–49.
28. Rants'o TA, Koekemoer LL, Panayides JL, Van RL. Potential of essential oil-based anticholinesterase insecticides against Anopheles vectors: A Review. Molecules, 2022; 27:2481–2484.
29. Enan EE. Molecular response of Drosophila melanogaster tyramine receptor cascade to plant essential oils. Insect Biochem and Mol Biol, 2005; 35: 309–321.
30. Regnault-roger C, Vincent C, Arnason JT. Essential oils in insect control: Low-risk products in a high-stakes world. Annual rev of Entomol, 2012; 57: 405–424.
31. Govindarajan M, Benelli G. Eco-friendly larvicides from Indian plants: effectiveness lavandulyl acetate and bicyclogermacrene on malaria, dengue and Japanese encephalitis mosquito vectors Marimuthu Govindarajan. Ecotox and Environ Safety, 2016;133:395–402.
32. Zhang R, Zhang W, Zheng J, Xu J, Wang H, Du J, Zhou D, Sun Y, Shen B. Toxic effects of *Perilla frutescens* (L.) Britt. essential oil and its main component on *Culex pipiens pallens* (Diptera: Culicidae). Plants, 2023; 12: 1516.
33. Mahanta S, Sarma R, Khanikor B. The essential oil of *Lippia alba* Mill (Lamiales: Verbenaceae) as mosquitocidal and repellent agent against *Culex quinquefasciatus* Say (Diptera: Culicidae) and *Aedes aegypti* Linn (Diptera: Culicidae). The J of Basic and Appl Zoology, 2019; 80: 64.
34. Bekele D. Review on insecticidal and repellent activity of plant Products for malaria mosquito control. Biomed Res and Rev, 2018; 2: 1–7.
35. Jemberie W, Tadie A, Enyew A, Debebe A, Raja N. Repellent activity of plant essential oil extracts against malaria vector *Anopheles arabiensis* Patton (Diptera: Culicidae). Entomon, 2016; 41: 91–98.
36. Solomon B, Gebre-Mariam T, Asres K. Mosquito repellent actions of the essential oils of *Cymbopogon citratus*, *Cymbopogon nardus* and *Eucalyptus citriodora*: Evaluation and formulation studies. J Ess Oil Bearing Plants, 2012;15: 766–773.
37. Dugassa S, Medhin G, Balkew M, Seyoum A, Gebre-michael T. Field investigation on the repellent activity of some aromatic plants by traditional means against *Anopheles arabiensis* and *An. pharoensis* (Diptera: Culicidae) around Koka, central Ethiopia. Acta Tropica, 2009; 112: 38–42.
38. Hadidy DE, Sayed E, Tantawy ME, Alfy TE, Farag S M, Haleem DRA. Larvicidal and repellent potential of *Ageratum houstonianum* against *Culex pipiens*. Scientific Rep, 2022; 12: 21410.
39. Liakakou A, Angelis A, Papachristos DP, Fokialakis N, Michaelakis A, Skaltsounis LA. Isolation of volatile compounds with repellent properties against *Aedes albopictus* (Diptera: Culicidae) using CPC technology. Molecules, 2021; 26: 3072.
40. Shoukat RF, Shakeel M, Rizvi SAH, Zafar J, Zhang Y, Freed S, Xu X, Jin F. Larvicidal, ovicidal, synergistic, and repellent activities of *Sophora alopecuroides* and its dominant constituents against

Aedes albopictus. Insects, 2020; 11: 246.

41. Shukla D, Wijayapala S, Vankar PS. Effective mosquito repellent from plant based formulation. Int J of Mosquito Res, 2018; 5: 19–24.
42. Alievi K, Capoani GT, Buzatto M, Miorando D, Serpa PZ, Fogolari O, Ignácio ZM, Simões DA, Busato MA, Lutinski JA, Junior WAR. *Ateleia glazioviana* and *Ocimum basilicum*: plants with potential larvicidal and repellent against *Aedes aegypti* (Diptera, Culicidae). Res, Soc and Devt, 2021;10: e228101724733.
43. Lim V, Mohd Narawi M, Chiu HI, Tung WH, Tan JJ, and Lee CK. Selected essential oils as repellents against *Aedes aegypti*: Validation of the bioconstituents using gas chromatography. J Essential Oil-Bearing Plants, 2019; 22: 1058–1073.
44. Al-Sarar S, Hussein HI, Abobakr Y, Al-Zabib AAS, Bazeyad AY. Mosquitocidal and repellent activities of essential oils against *Culex Pipiens* L. Entomo Res, 2020; 50:182–188.
45. Yusuf Y, Efendi K, Diantasari S. Larvicidal activity test of ethanolic extract of (*Euphorbia tirucalli* Linn) stem on *Aedes aegypti* larvae. Syst Rev in Pharmacy, 2020; 11: 388–392.

Figures

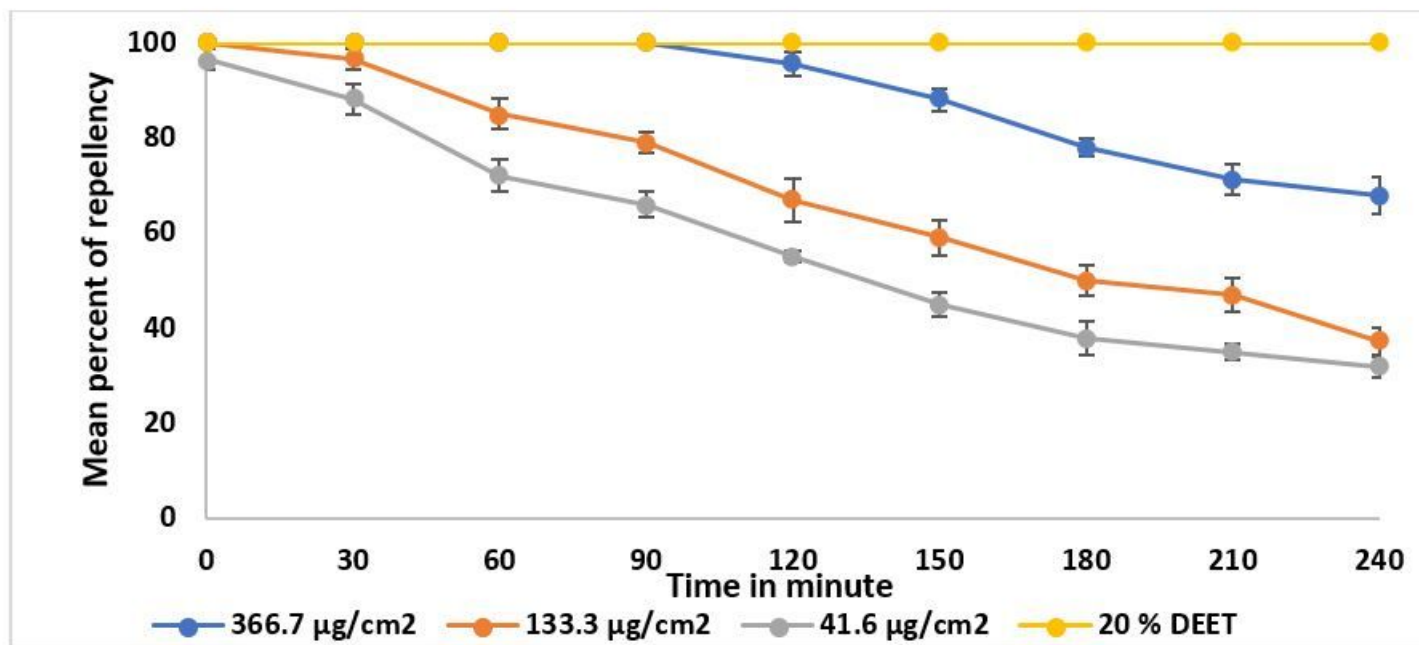


Figure 1

Mean percentage repellent activity of essential oil from the leaf of *Leucas stachydiformis* against *Anopheles arabiensis* at different concentrations