

Larvicidal activities of column chromatographic fractions of an ethanol leaf extract from *Ricinus communis* against *Anopheles arabiensis* Patton (Diptera: Culicidae)

Araya Eukubay^{1*}, Emana Getu², Eyob Debebe¹ and Mamuye Hadis¹

¹Ethiopian Public Health Institute, Addis Ababa, Ethiopia

²Department of Zoological Sciences, Addis Ababa University, Addis Ababa, Ethiopia

*Corresponding author: Email: eukubay.ephi2007@gmail.com Phone number: +251920868300/75337101

Abstract

Background: Long-term use of insecticide-based interventions for malaria control has resulted in the development of behavioral, physiological, and genetic resistance of the *Anopheles* mosquitoes. This calls for the development of sustainable, effective, and eco-friendly vector control measures. This study aimed to evaluate the bioactivity of column chromatographic fractions of ethanol leaf extract of *Ricinus communis* against *Anopheles arabiensis* larvae.

Methods: Dried leaves of the test plant were ground to powder and subjected to maceration using 80% ethanol for 72 hours. The crude extract was further fractionated with column chromatography using solvent systems of gradually increasing polarity. All the fractions were analyzed with thin-layer chromatography. Eluents having similar constituents were pooled and tested for their larvicidal potential against laboratory-reared susceptible *An. arabiensis* at a concentration ranging from 50ppm to 250ppm following WHO protocol.

Results: Fractions F1, F2, F3, and F5 demonstrated larval mortalities ranging from 34.67% to 100% in all the test concentrations while no larval mortalities were observed for fractions F9, F10, and F11 in all the test concentrations. The highest (100%) larval mortalities were observed for F2 and F3 at 100 to 250 ppm with the LC₅₀ of 21.012 and 28.410 ppm, respectively.

Conclusion: Fractions F2 and F3 caused 100% larval mortality at 100 to 250 ppm concentration with the lowest LC₅₀ of 21.012 and 28.410 ppm, respectively, suggesting their inputs for the development of botanical insecticides.

Keywords: *Anopheles arabiensis*; larvicidal activities; plant extract; *Ricinus communis*; column chromatography

Introduction

Vector-borne diseases exert a huge burden of morbidity and mortality worldwide, particularly affecting the poorest of the poor (Wilson et al. 2020). Malaria is one of the vector-borne diseases transmitted by the bite of mosquitoes belonging to the genus *Anopheles* (WHO 2019a). Despite the massive investment in intervention measures, malaria is still a major public health problem in Sub-Saharan countries (Afrane et al. 2016). In 2019, 229 million cases of malaria were reported worldwide and most of the cases (94%) were from Sub-Saharan Africa (WHO 2019b). *Anopheles arabiensis*, *An. coluzzii*, *An. gambiae* from the gambiae complex, and *An. funestus* from the Funestus subgroup are the most important vectors responsible for the transmission of both *Plasmodium falciparum* and *P. vivax* parasites to humans in Africa (Wiebe et al. 2017).

Vector control is the main strategy to mitigate the disease. Most national malaria control programs in Africa rely on indoor residual spraying (IRS) and using long-lasting insecticidal nets (LLINs) to reduce malaria incidence (Beier et al. 2018). However, these insecticide-based control interventions are generally not effective and face many challenges. Insecticide resistance, shifts in mosquito biting and resting behavior (biting outdoors and/or biting indoors and

leaving the house before getting a lethal dose of insecticide), and changes in human behavior such as sleeping outdoors during night time are some of the challenges that limit the effectiveness of these two core vector control interventions (Killeen 2014; Abraham et al. 2017; WHO 2018). Hence, there is a need for the development of sustainable, effective, and eco-friendly control measures, which target the immature stages of the vectors to circumvent both the insecticide and behavioral resistance problems found in all stages of mosquitoes.

Larval control is a proven control intervention as it targets the immature stages before they leave the aquatic habitats (Derua et al. 2019). Conventional larvicides such as temephos, fenitrothion, and diflubenzuron have been applied to target larval mosquitoes (WHO 2013). However, the use of these synthetic insecticides raises environmental concerns (Kim et al. 2020). As a result, interest in the use of natural plant products as an alternative mosquito control measure is growing (Asadollahi et al. 2019; Falkowski et al. 2020; Pratheeba et al. 2019; Silvério et al. 2020; Vivekanandhan et al. 2018; Xu et al. 2020). Compounds of plant origin have many advantages as they are cost-effective, environmentally friendly, biodegradable, and safe for non-target organisms (Rossi et al. 2020; Senthil-Nathan 2020).

Several studies have shown that plant extracts have promising mosquitocidal activities against malaria vectors (Ali and Venkatesalu 2020; Egunjobi and Okoye 2020; Eukubay et al. 2021; Krishnappa et al. 2020). *Ricinus communis*, which belongs to family Euphorbiaceae, is claimed to have medicinal importance (Franke et al. 2019). In addition, crude extracts of the plant have shown potential larvicidal activities against *An. arabiensis*, *An. culicifacies*, and *Aedes aegypti* (Eukubay et al. 2021; Sogan et al. 2018). Crude plant extracts that have promising insecticidal activities have to be further purified and tested to identify their active compounds and produce effective botanical insecticides. Thus, the current study aimed to investigate the larvicidal activities of column chromatographic fractions of ethanolic leaf extract of *R. communis* against *An. arabiensis*.

Materials and Methods

Plant collection and preparation of crude extracts:

Leaves of *R. communis* were collected on 01 December 2018 from the Ethiopian Public Health Institute-Traditional and Modern Medicine Directorate's garden, which is located at 9° 02' 52" N, 38° 43' 50" E. The plant was identified by expert from the National Herbarium of Addis Ababa University and voucher specimens were deposited at the National Herbarium with AE-02 specimen number. The leaves were washed thoroughly and air-dried under shade at room temperature (25-27°C). Dried leaves were ground to powder using a grinding mill (Christy and Norries Ltd. England). Three hundred grams of leaf powder was macerated with 80% ethanol in 1:10 (W/V) using Erlenmeyer flasks and placed on an orbital shaker (Gallenkamp 5A-4131. England) at room temperature for 72 hours (Tomass et al. 2011; Eukubay et al. 2021). The filtrate was concentrated using a rotary evaporator (Buchi RE 121, Switzerland) at 40°C to remove solvent from the extract. The crude extract was then collected in small volume beakers and kept in a water bath at 40°C until the solvent remaining from the rotary evaporator was completely evaporated.

Thin layer chromatography: Crude ethanol leaf extract was analyzed using thin-layer chromatography (TLC) (Merck, 60F254) to standardize a suitable solvent system. Silica gel, 20x20 cm, 0.20 mm thick, cut into 5x15 cm was used. Hexane, ethyl acetate, ethanol, and methanol were used as the mobile phase. The following ratios of solvent combinations were sequentially used in the analysis using the TLC: Hexane:/ Ethyl acetate 8:2, 6:4, 4:6, 2:8; Ethyl acetate:/ Ethanol 8:2, 6:4, 4:6, 2:8; and Ethanol:/ Methanol 8:2, 6:4, 4:6, 2:8. The TLC analysis with the above solvent systems showed that hexane-and ethyl acetate in a ratio of 4:6 gave the most pronounced separation

with four distinct spots and was selected as the best solvent system.

Column chromatographic fractionation of the crude leaf extract:

The crude leaf extract was fractionated using column chromatography using silica gel 60 (0.063-0.2mm mesh size). Column chromatographic elution of crude leaf extract was done with solvent systems of gradually increasing polarity using hexane, ethyl acetate, ethanol, and methanol. The ratios of solvent combinations were sequentially used in the elution process. Hexane: ethyl acetate 100:0, 80:20, 60:40, 40: 60, and 20: 80; ethyl acetate: ethanol 100:0, 80:20, 60:40, 40: 60, 20: 80 and 0:100; and ethanol: methanol 80:20, 60:40, 40: 60, 20: 80 and 0:100. Known volume of each solvent combination was sprayed using a 10 ml syringe by the sides of the glass into the column each time. This measure prevents solvent droplets from falling directly and disturbing the topmost layer of the column as a distortion of this layer would result in a non-uniform drain of the fractions (Eukubay et al. 2021). Column chromatographic eluents were collected in separate flasks and examined by thin-layer chromatography (TLC). This was done on silica gel plates (Merck, 60 F254) using Hexane/Ethyl acetate in a 4:6 ratio as a mobile phase. Visualization and identification of spots that indicate constituents of each eluent were done using an Ultra Violet lamp at a wavelength of 254 nm. Finally, eluents having similar constituents were pooled and concentrated using a rotary evaporator at 38-40°C and placed in a deep freezer until used for mosquito larvicidal tests.

Preparation of test and control solutions for chromatographic fractions:

Two hundred milligrams of each fraction were dissolved in two mL of ethanol and made up to 20 mL with distilled water using standard measuring flasks to obtain 20 mL of 1% stock solution. From the stock solution, concentrations of 50, 100, 150, 200, and 250 ppm were prepared by adding the appropriate volume of dilution. Tween 80 (0.1 mL) was used as an emulsifier. A mixture of two mL of ethanol and 0.1 mL of Tween 80 was diluted to 100 mL of distilled water in a standard measuring flask to serve as a negative control (WHO 2005).

Mosquito rearing: Susceptible laboratory colonies of *An. arabiensis* were obtained from the Ethiopian Public Health Institute (EPHI) Insectary. Mosquitoes were reared using WHO standard procedures (WHO 2005; WHO 2013). They were maintained at 25±2°C and 80±10% relative humidity and dark: light (12:12hrs). Larvae were fed with tetramin fish food. Late-third instar larvae were used for all the tests (WHO 2013).

Larvicidal bioassays: Each fraction was tested against 3rd instar *An. arabiensis* larvae following procedures described by WHO (WHO 2005). Batches of 25 late third instar larvae of *An. arabiensis* were transferred using droppers to 200 mL glass beakers each containing 100 mL of water. Small, unhealthy, or damaged larvae were removed and replaced. The appropriate volume of dilution was added to 100 mL of water in the beakers to obtain the desired target dosage. Three test replicates alongside a negative control were used for each concentration. The test containers were held at 25±2°C and 80± 10% relative humidity with a photoperiod of 12 hrs light followed by 12 hrs dark. Larval mortality was recorded after 24 hours of exposure in each concentration of test solutions. Larvae were confirmed dead when they failed to move after probing them with a needle at their cervical region.

Data analysis: Mean percent mortalities after 24 hours exposure of the third instar larvae of *An. arabiensis* were calculated and two way analysis of variance (ANOVA) was conducted using SPSS version 20. Tukey HSD test was used to separate significant means ($p < 0.05$) in larval mortalities among different fractions and between concentrations of each fraction. The LC₅₀ and LC₉₀ at 95% fiducial limits of upper confidence limit and lower confidence limit and chi-square values were determined by probit regression analyses using SPSS program version 20 to determine their larvicidal efficacies. The level of significance for the statistical analysis was set at $P < 0.05$.

Table 2: Mean percent mortality of 3rd instar *Anopheles arabiensis* larvae after 24 h exposures to the different column chromatographic fractions of ethanol leaf extract of *Ricinus communis*.

	Mean± SE Concentration					
Fractions	50 ppm	100 ppm	150 ppm	200 ppm	250 ppm	Control
F1	34.67±2.67 ^{aA}	58.67±1.33 ^{aB}	80.00±0.00 ^{aC}	81.33±1.33 ^{aC}	93.33±3.53 ^{aD}	0.00±0.00 ^{Ea}
F2	87.00±1.33 ^{bA}	100.00±0.00 ^{bB}	100.00±0.00 ^{bB}	100.00±0.00 ^{bB}	100.00±0.00 ^{bB}	0.00±0.00 ^{Ca}
F3	81.33±3.53 ^{bA}	100.00±0.00 ^{bB}	100.00±0.00 ^{bB}	100.00±0.00 ^{bB}	100.00±0.00 ^{bB}	0.00±0.00 ^{Ca}
F4	0.00±0.00 ^{dA}	66.67±3.52 ^{cB}	89.33±2.67 ^{cC}	100.00±0.00 ^{bD}	100.00±0.00 ^{bD}	0.00±0.00 ^{Aa}
F5	69.33±1.33 ^{cA}	85.33±1.33 ^{dB}	97.33±1.33 ^{bC}	100.00±0.00 ^{bC}	100.00±0.00 ^{bC}	0.00±0.00 ^{Da}
F6	0.00±0.00 ^{dA}	0.00±0.00 ^{eA}	21.33±1.33 ^{dB}	73.33±1.33 ^{cC}	100.00±0.00 ^{bD}	0.00±0.00 ^{Aa}
F7	0.00±0.00 ^{dA}	0.00±0.00 ^{eA}	0.00±0.00 ^{eA}	42.67±1.33 ^{dB}	53.33±1.33 ^{cC}	0.00±0.00 ^{Aa}
F8	0.00±0.00 ^{dA}	0.00±0.00 ^{eA}	0.00±0.00 ^{eA}	38.67±3.53 ^{dB}	61.33±1.33 ^{dC}	0.00±0.00 ^{Aa}
F9	0.00±0.00 ^{dA}	0.00±0.00 ^{eA}	0.00±0.00 ^{eA}	0.00±0.00 ^{eA}	0.00±0.00 ^{eA}	0.00±0.00 ^{Aa}
F10	0.00±0.00 ^{dA}	0.00±0.00 ^{eA}	0.00±0.00 ^{eA}	0.00±0.00 ^{eA}	0.00±0.00 ^{eA}	0.00±0.00 ^{Aa}
F11	0.00±0.00 ^{dA}	0.00±0.00 ^{eA}	0.00±0.00 ^{eA}	0.00±0.00 ^{eA}	0.00±0.00 ^{eA}	0.00±0.00 ^{Aa}

*Means followed by the same letters within the same row (Upper case) and within the same column (Lower case) are not significantly different ($p > 0.05$)

At the lowest concentration (50ppm), only F1, F2, F3 and F5 showed larvicidal activities with a percent larval mortality of 34.67%, 87%, 81.33%, and 69.33, respectively. F2 and F3 demonstrated 100% larval mortalities at 100 to 250ppm concentrations while 100% mortalities for F4 and F5 were achieved at 200

Results

Column chromatography fractions of ethanol leaf extract of *Ricinus communis*: Twenty-nine fractions were collected during the column chromatographic fractionation of ethanol leaf extract of *R. communis*. Fractions with similar TLC profiles were combined and designated as F1 to F11 (Table 1).

Table 1: Column chromatography fractions of ethanol leaf extract of *Ricinus communis*

Fractions collected	Designated as
Fraction 1	F1
Fraction 2	F2
Fraction 3	F3
Fraction 4	F4
Fractions 5-7	F5
Fractions 8-12	F6
Fractions 13-16	F7
Fractions 17-21	F8
Fractions 22-24	F9
Fractions 25-27	F10
Fractions 28-29	F11

Larvicidal activities of column chromatographic fractions of ethanol leaf extract of *R. communis* against *An. arabiensis* larvae: Each fraction was tested separately against 3rd instar *An. arabiensis* larvae and mean percent mortality after 24 h exposures are given in Table 2.

to 250ppm. F6 performed 100% larval mortalities at the two highest concentrations (200 and 250 ppm). Furthermore, no larval mortalities were observed for F9 to F11 in all concentrations and the negative controls.

Statistical analysis of variance (ANOVA) revealed that no significant ($P>0.05$) larval mortalities were observed between F2 and F3 in all the test concentrations (Table 2). In addition, no significant ($P>0.05$) larval mortalities were observed for F1 and F2 among the test concentrations of 100 to 250 ppm. However, significant ($P<0.05$) larval mortalities were observed in F1, F4, and F5 at 50, 100, and 150ppm concentrations.

The percentage mortalities of fractions F1 to F8 were subjected to probit analysis to determine the dose-

response against 3rd instar *An. arabiensis* larvae and the results are presented in Table 3. F2, F3, and F5 demonstrated the highest percent larval mortalities with the lowest concentrations (LC_{50} = 21.012, 28.410, 36.800ppm and LC_{90} = 54.527, 61.850, and 96.020ppm, respectively). While the relatively lowest percent larval mortalities were observed for F7 to F8 with LC_{50} = 231.578, 226.646, and LC_{90} =321.932, 301.002ppm, respectively. The results indicated that larval mortality increases as the test concentrations increase.

Table 3: LC_{50} and LC_{90} of column chromatographic fractions of *Ricinus communis* leaf extract induced percent mortalities of *Anopheles arabiensis* larvae

Fractions	LC_{50} (ppm)	LCL	UCL	LC_{90} (ppm)	LCL	UCL	df ^b	X^2
F1	75.139	63.402	85.687	245.602	208.322	308.845	3	3.518
F2	21.012	7.270	31.221	54.527	40.659	66.670	3	2.476
F3	28.410	15.768	36.811	61.850	52.490	73.739	3	2.416
F4	93.802	71.659	110.864	137.343	115.828	193.548	3	9.610
F5	36.800	26.891	45.000	96.020	84.024	112.512	3	6.076
F6	174.638	168.833	180.327	217.952	208.920	230.388	3	3.858
F7	231.578	190.097	1323.503	321.932	253.723	658553.28	3	17.774
F8	226.646	201.602	278.590	301.002	256.113	611.463	3	9.698

* LC_{50} = Lethal concentration that kills 50% of the exposed larvae, LC_{90} = Lethal concentration that kills 90% of the exposed larva, UCL=Upper confidence limit, LCL= Lower confidence limit, X^2 = Chi-square, df= Degree of freedom

Discussion

In this study, fractions F1, F2, F3, and F5 demonstrated larval mortalities ranging from 34.67% to 100% in all the test concentrations while no larval mortalities were observed for fractions F9, F10, and F11 in all the test concentrations. This suggests that the bioactive compounds are confined to F1, F2, F3, and F5 fractions. A similar study on larvicidal activities of seven (F1 to F7) column chromatographic fractions of methanol leaf extract of *Calpurnia aurea* revealed that fractions F1, F2, and F3 performed better larvicidal activities with percent larval mortalities against *An. arabiensis* larvae (Eukubay et al. 2021).

Fractions F2 and F3 showed the highest larvicidal activities with LC_{50} of 21.012 and 28.410 ppm, respectively, and 100% larval mortalities at 100-250 ppm concentration. A previous study (Basheer 2014) on the larvicidal activity of column chromatographic fractions of ethyl acetate leaf extract of *R. communis* found that fraction F3 displayed 100% mortality at 86 ppm with an LC_{50} of 125 ppm against *An. arabiensis*. The difference in LC_{50} (larvicidal activity) with the current study could be due to the difference in the extraction solvent and the geographical origin of the test plant. The amount and type of phytochemicals extracted from plants are determined by the type of solvent used and extraction methods (Pandey and Tripathi 2014). In addition, it is also reported that the concentration and type of bioactive molecules are

dependent on species genotype, physiology, and other environmental factors during their growth (Isah 2019). The study on the larvicidal potential of crude ethanol leaf extract of *R. communis* against *An. arabiensis* also revealed that 93.33% larval mortality was achieved at 300 ppm with the LC_{50} of 134.03 ppm (Eukubay et al. 2021) while in the current study where the same crude plant extract was fractionated and tested for its larvicidal activity, 100% larval mortality was achieved at 100 ppm with LC_{50} of 21.012 and 28.410 ppm for F1 and F2 fractions, respectively. This result indicated the use of the active fractions for larvicide against *An. arabiensis* would help to get 100% mortality at the lowest dosage than the use of crude extracts.

Limitations of the study

It was unable to identify the bioactive compounds of the fractions with higher larvicidal activities due to financial constraints. Furthermore, data on retardation factor (Rf), and percent yield of the fractions are not included in the analysis.

Conclusion

The current study revealed that column chromatographic fractions F1 to F8 of ethanol leaf extract of *R. communis* demonstrated larvicidal potential against *An. arabiensis* larvae while F9 to F11 showed no activity. Fractions F2 and F3 performed better larvicidal activity with LC_{50} of 21.0 and 28.4 ppm, respectively, and 100% larval mortalities at 100-

250 ppm concentration than the rest. This finding suggests that the two fractions have the potential for the production of botanical-based insecticides provided that further studies are conducted to identify the compounds of F1 and F2 fractions and evaluate their efficacy under field conditions.

Funding: The research received financial support from Addis Ababa University, College of Natural and Computational Sciences, Department of Zoological Sciences (Insect Sciences).

Conflict of interest: The authors declare that they have no competing interest in this work.

Acknowledgments

The authors are grateful to the Department of Zoological Sciences of Addis Ababa University for financial support. Traditional and Modern Medicine Research Directorate, and Public Health Entomology Research Team of Ethiopian Public Health Institute – are also acknowledged for their support with the required equipment and access to the laboratory. Mr. Fitsum Tesfaye, Mrs. Genet Nigatu, and Mrs. Asnaku Adera are acknowledged for their assistance in mosquito rearing.

References

- Abraham M, Massebo F & Lindtjørn B (2017). High entomological inoculation rate of malaria vectors in an area of high coverage of interventions in southwest Ethiopia: Implication for residual malaria transmission. *Parasite Epidemiology and Control*, 2(2): 61–69.
- Afrane YA, Bonizzoni M & Yan G (2016). Secondary malaria vectors of Sub-Saharan Africa: a threat to malaria elimination on the continent? *INTECH*://doi.org/10.5772/65359.
- Ali SI & Venkatesalu V (2020). Larvicidal activity of *Neolamarckia cadamba* against the *Anopheles stephensi*, *Aedes aegypti*, and *Culex quinquefasciatus*. *Proceedings of Zoological Society*, 73(3): 227–234.
- Asadollahi A, Khoobdel M, Zahraei-Ramazani A, Azarmi S & Mosawi SH (2019). Effectiveness of plant-based repellents against different *Anopheles* species: A systematic review. *Malaria Journal*, 18(1): 1–20.
- Basheer AGM (2014). *Ricinus communis* (Castor) as larvicide on *Anopheles arabiensis* Patton. *International Journal of Advance Pharmacy, Biology and Chemistry*, 3(2): 319–328.
- Beier C, Wilke ABB & Benelli G (2018). Newer approaches for malaria vector control and challenges of outdoor transmission. *Intech* 387–402.
- Derua YA, Kweka EJ, Kisinza W, Githeko AK & Mosha FW (2019). Bacterial larvicides used for malaria vector control in sub-Saharan Africa: a review of their effectiveness and operational feasibility. *Parasites & Vectors* 12: 426.
- Egunjobi FB & Okoye IC (2020). Ovicidal and larvicidal activities of ethanolic leaf extracts of three botanicals against the malaria vector - *Anopheles gambiae*. *International Annals of Science*, 9(1): 111–121.
- Eukubay A, Getu E, Debebe E & Hadis M (2021). Larvicidal potential of some plant extracts against *Anopheles arabiensis* Patton (Diptera: Culicidae). *International Journal of Tropical Insect Sciences*, 41: 479–485.
- Falkowski M, Jahn-Oyac A, Odonne G, Flora C, Estevez Y, Touré, S, et al. (2020). Towards the optimization of botanical insecticides research: *Aedes aegypti* larvicidal natural products in French Guiana. *Acta Tropica*, 201: 105179.
- Franke H, Scholl R & Aigner A (2019). Ricin and *Ricinus communis* in pharmacology and toxicology-from ancient use and “*Papyrus Ebers*” to modern perspectives and “poisonous plant of the year 2018. *Naunyn Schmiedebergs Archives of Pharmacology*, 392(10): 1181–1208.
- Isah T (2019). Stress and defense responses in plant secondary metabolites production. *Biological Research*, 52: 39.
- Killeen GF (2014). Characterizing, controlling, and eliminating residual malaria transmission. *Malaria Journal*, 13(1): 1–22.
- Kim SH, Bae JW, Kim DH, Jeong DJ, Ha JJ, Yi JK, et al. (2020). Detrimental effects of temephos on male fertility: An in vitro study on a mouse model. *Reproductive Toxicology*, 96: 150–155.
- Krishnappa K, Baranitharan M, Elumalai K & Pandiyan J (2020). Larvicidal and repellent effects of *Jussiaea repens* (L.) leaf ethanol extract and its major phytoconstituents against important human vector mosquitoes (Diptera: Culicidae). *Environmental Sciences and Pollution Research*, 27(18): 23054–23061.
- Pandey A & Tripathi S (2014). Concept of standardization, extraction, and pre-phytochemical screening strategies for the herbal drug. *Journal of Pharmacognosy Phytochemistry*, 2(5): 115–119.
- Pratheeba T, Vivekanandhan P, Faeza AKN & Natarajan D (2019). Chemical constituents and larvicidal efficacy of *Naringi crenulata* (Rutaceae) plant extracts and bioassay-guided fractions against *Culex quinquefasciatus* mosquito (Diptera: Culicidae). *Biocatalysis and Agricultural Biotechnology*, 19: 101137.
- Rossi P, Cappelli A, Marinelli O, Valzano M, Pavoni L, Bonacucina G, et al. (2020). Mosquitocidal and anti-inflammatory properties of the essential oils

- obtained from Monoecious, male, and female inflorescences of Hemp (*Cannabis sativa* L.) and Their encapsulation in nanoemulsions. *Molecules*, 25: 3451.
- Senthil-Nathan S (2020). A review of resistance mechanisms of synthetic insecticides and botanicals, phytochemicals, and essential oils as alternative larvicidal agents against mosquitoes. *Frontiers in Physiology*, 10: 1–21.
- Silvério MRS, Espindola LS, Lopes NP & Vieira PC (2020). Plant natural products for the control of *Aedes aegypti*: The main vector of important arboviruses. *Molecules*, 25: 3484.
- Sogan N, Kapoor N, Singh H, Kala S, Nayak A & Nagpal, BN (2018). Larvicidal activity of Ricinus communis extracts against mosquitoes. *Journal of Vector-Borne Diseases*, 55: 282–290.
- Tomass Z, Hadis M, Taye A & Mekonnen Y, Petros B (2011.). Larvicidal effects of *Jatropha curcas* L. against *Anopheles arabiensis* (Diptera : Culicidae). *Momona Ethiopian Journal of Sciences*, 3: 52-64.
- Vivekanandhan P, Venkatesan R, Ramkumar G & Karthi S (2018). Comparative analysis of major mosquito vectors' response to seed-derived essential oil and seed pod-derived extract from *Acacia nilotica*. *International Journal of Environmental Research and Public Health*, 15(2): 388.
- WHO (2005). Guidelines for laboratory and field testing of mosquito larvicides. World Health Organization, Geneva.
- WHO (2013). Malaria entomology and vector control: Participants guide. World Health Organization, Geneva.
- WHO (2018). Global report on insecticide resistance in malaria vectors: 2010–2016. World Health Organization, Geneva.
- WHO (2019a). Guidelines for malaria vector control. World Health Organization, Geneva.
- WHO (2019b). World malaria report. World Health Organization, Geneva.
- Wiebe A, Longbottom J, Gleave K, Shearer FM, Sinka ME, Massey NC, et al. (2017). Geographical distributions of African malaria vector sibling species and evidence for insecticide resistance. *Malaria Journal*, 16: 85
- Wilson IAL, Courtenay O, Kelly-hope LA, Scott TW, Takken W, Torr SJ, et al. (2020). The importance of vector control for the control and elimination of vector-borne diseases. *PLoS Neglected Tropical Diseases*, 14 (1): e0007831. <https://doi.org/10.1371/journal.pntd.0007831>.
- Xu Y, Qin J, Wang P, Li Q, Yu S, Zhang Y, et al. (2020). Chemical composition and larvicidal activities of essential oil of *Cinnamomum camphora* (L.) leaf against *Anopheles stephensi*. *Revista da Sociedade Brasileira de Medicina Tropical*, 53: e20190211 doi: 10.1590/0037-8682-0211-2019.