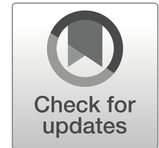
International Journal of Tropical Insect Science (2021) 41:479–485
https://doi.org/10.1007/s42690-020-00229-1

ORIGINAL RESEARCH ARTICLE



Larvicidal potential of some plant extracts against *Anopheles arabiensis* Patton (Diptera: Culicidae)

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Received: 7 March 2020 / Revised: 21 July 2020 / Accepted: 31 July 2020 / Published online: 10 August 2020
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Abstract

Insecticide based vector control intervention is threatening with the emergence and spread of resistant vectors. The development of other eco-friendly and cost effective vector control tools is required to alleviate this problem. In this study, five selected plant leaf extracts were evaluated for their larvicidal activities against 3rd instar *An. arabiensis* larvae at concentrations ranging from 50 to 300 ppm following the WHO protocol. The best performing extract was subjected to bioassay-guided column chromatographic fractionation and all fractions were tested separately for their larvicidal activities. All the plant extracts were found to have potential larvicidal activities against 3rd instar *An. arabiensis* larvae. Methanol crude extract of *Calpurnia aurea* achieved 100% larval mortality at 300 ppm with LC_{50} of 84.85 ppm while less activity was observed for methanol crude extract of *Artemisia annua*, 68% larval mortality at 300 ppm. Column chromatographic fractions F1and F3 of methanol extract of *C. aurea* caused 100% mortality at 250 and 300 ppm with LC_{50} of 62.51 and 82.33 ppm, respectively. The present study revealed that column chromatographic fractions F1 and F3 of methanol extract of *C. aurea* caused a remarkable larvicidal activity against *An. arabiensis* demonstrating a high potential for botanical mosquito insecticide development.

Keywords Diptera · Culicidae · *Anopheles arabiensis* · Botanical larvicide · *Calpurnia aurea* · Column chromatography

Introduction

Mosquitoes are vectors of several medically important pathogens and parasites that cause serious human diseases such as malaria, dengue, yellow fever, chikungunya and encephalitis or filariasis (Becker et al. 2010). Human malaria is transmitted by female mosquitoes of genus *Anopheles*. In Africa *An. gambiae*, *An. coluzzii*, *An. arabiensis* and *An. funestus* are the major malaria vectors (Sinka et al. 2012). Vector control is a crucial prevention tool to mitigate mosquito borne diseases. Long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) are the primary malaria prevention strategies in many African countries (Hemingway et al. 2018). However, this synthetic insecticide based control methods are threatened with the development of resistance among malaria vectors (Sougoufara et al. 2017; WHO 2018) and have a negative adverse effect on humans and the environment (Chrustek et al. 2018). With this regard, scientists are investigating botanical insecticides for sustainable, healthier and eco-friendly vector control methods (Vivekanandhan and Senthil-nathan 2017; Vivekanandhan et al. 2018; Pavela et al. 2019). Botanical insecticides are plant derived compounds that have insecticidal properties (Pratheeba et al. 2019). They are selective, biodegradable, and have minor or no adverse effects on non-target organisms and the environment (Kweka et al. 2016) though some exceptions could be toxic to humans and animals (Welch et al. 2018). This makes natural plant products to be potentially appropriate for use in integrated vector management programs.

Larval control of malaria vector is a proven preventive method as it targets the immature stages before they escape from the aquatic habitats (Derua et al. 2019). Temephos, Fenthion, Diflubenzuron and Pyriproxyfen are some of the compounds recommended for mosquitoes larvicides (WHO 2013). Applying these conventional larvicides in the water sources, however, causes many risks to humans and/or the environment. The use of natural larvicides derived from plants are more promising in this aspect (Vivekanandhan et al.

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2019). Ethiopia is endowed with several medicinal plants and the majority of the population used these plants to treat a number of diseases (Alebie et al. 2017). *Calpurnia aurea* (Aiton), (Fabaceae) is one of the medicinal plants used to treat different human and animal diseases. Extract of this plant is used as a remedy of different diseases and to control ticks, lice and maggot- infested wounds (Zorloni et al. 2010). However, its biological activity against mosquitoes has not been tested. *Artemisia annua* L. of the family Asteraceae is also medicinal plant used to treat malaria parasite and is also reported to have larvicidal activities against *Aedes aegypti*, *An. sinensis* and *Culex quinquefasciatus* (Cheah et al. 2013) but its larvicidal activity against *An. arabiensis* is not determined. In addition, plants such as *Clausena anisata* (Wild) Hook. F.ex. (Rutaceae), *Datura stramonium* L. (Solanaceae), and *Ricinus communis* L. (Euphorbiaceae) are traditionally used as insect bite repellents (Karunamoorthi and Hailu 2014). Literature also reveals that these plants have larvicidal activities against *Cx. quinquefasciatus, Ae. aegypti* and *An. arabiensis* in different countries (Elimam et al. 2009; Swathi et al. 2010; Mavundza et al. 2013; Okbatinsae and Haile 2017). Exploring plant species that adapt to different geographical and environmental factors is a crucial effort to control disease vectors. Thus, this study focused on the evaluation of larvicidal activity of the aforementioned plant leaf extracts against *An. arabiensis* under laboratory conditions.

Materials and methods

Collection of test plants

The leaves of test plants were collected from different areas of Ethiopia; *R. communis* and *D. stramonium* from around Addis Ababa located at 9^0 02' 52" N, 38^0 43’ 50"E and 8^0 57' 48" N, 38^0 43' 18" E, respectively. *C. aurea* was collected from Wondogenet, southern Ethiopia (7^0 06' 07" N, 38^0 37' 35" E), *C. anisata* from Menagesha, central Ethiopia (9^0 03' 19" N, 38^0 33' 37" E) and *A. annua* from Chencha District, southern Ethiopia (6^0 15' 03" N, 37^0 34' 48" E. The plants were identified by National Herbarium of Addis Ababa University and voucher specimens were deposited at the National Herbarium with a voucher number of AE- 01 (*C. anisata*), AE-02 (*R. communis*), AE-03 (*D. stramonium*), AE-04 (*C. aurea)* and AE-05 (*A. annua*).

Preparation of crude leaf extracts

Plant extraction was conducted in Traditional and Modern Medicine Research Directorate laboratory of the Ethiopian Public Health Institute (EPHI). The leaves were washed thoroughly and air-dried under shade at room temperature (25–27 ^{0}C). Dried leaves were ground to powder separately using grinding mill (Christy and Norries Ltd. England). 300 g of each plant leaf powder was macerated with 80% ethanol and methanol in 1:10 (W/V) using Erlenmeyer flasks and placed on orbital shaker (Gallenkamp 5A-4131. England) at room temperature for 72 h (Tomass et al. 2011). The leaf extract of *C. aurea*, *C. anisata*, *R. communis*, *D. stramonium* and *A. annua* were filtered through cotton and subsequently with Whatman filter paper (12.5 cm size). Filtrates were concentrated using rotary evaporator (Buchi RE 121, Switzerland) at 40 ^{0}C to remove solvent from the extracts. The crude extracts were then collected in small volume beakers and further concentrated on water bath at 40^{0}C and then stored in deep freeze until used in mosquito larvicidal tests.

Preparation of test and control solutions

Two hundred milligrams of the dried crude ethanol extract was dissolved in 2 mL of ethanol and made up to 20 mL with distilled water using standard measuring flask to prepare 20 mL of 1% stock solution. The same amount of methanol crude extract was also dissolved in 20 mL distilled water to obtain 1% stock solution. From the stock solution, concentrations of 50, 100, 150, 200, 250 and 300 ppm were prepared by adding the appropriate volume of dilution. 0.1 mL of Tween 80 was used as an emulsifier. A mixture of 2 mL of ethanol and 0.1 mL of Tween 80 (for 80% ethanol extract) and 0.1 mL of Tween 80 (for methanol extract) were made up to 100 mL in a standard measuring flask by adding distilled water to serve as the negative control solution (WHO 2005).

Mosquito rearing

Susceptible colonies of *An. arabiensis* were obtained from insectary at the Ethiopian Public Health Institute (EPHI). Mosquitoes were reared using WHO standard procedures (WHO 2005, 2013). They were maintained at 25 ± 2 ^{0}C and $80 \pm 10\%$ relative humidity and 12:12 light and dark photoperiod. Larvae were fed with Tetramin fish food. Late third instar larvae were used for all the tests.

Larvicidal bio-assay with crude leaf extract

In the first phase of bio-assay the larvicidal activities of ethanol and methanol crude leaf extracts of *A. annua*, *C. aurea*, *C. anisata*, *D. stramonium* and *R. communis* were screened against 3rd instar larvae of *An. arabiensis* at 300 ppm concentration. Larvicidal bioassay was conducted for 24 h in glass beakers of 100 mL test solutions with three replicates following WHO guideline (WHO 2005). Batches of 25 late third instar larvae of *An. arabiensis* were transferred by means of 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. Each test was run three times on different days. The test containers were held at 25 ± 2^0C and $80 \pm 10\%$ relative humidity with a photoperiod of 12 h light followed by 12 h dark. Larval mortality was recorded after 24 h 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.

Based on the performance of preliminary screening leaf extracts that caused 90% and above larval mortality at 300 ppm were selected and subjected for dose-response bioassay at the concentrations of 50, 100, 150, 200, 250 and 300 ppm. The average mortality after 24 h exposure were recorded and used to determine LC_{50} and LC_{90} values.

Thin layer chromatography (TLC)

The crude extracts were firstly analyzed using the TLC (Merck, 60F254) to establish suitable solvent system (silica gel, 20 × 20 cm, 0.20 mm thick, cut into 5 × 15 cm for use). The main solvents used as the mobile phase were hexane, ethyl acetate and methanol. The following ratios of solvent combinations were sequentially used in the analysis using the TLC ; Hexane: ethyl acetate 10:0, 8:2, 7:3, 6:4, 4: 6, 3:7, 2: 8; ethyl acetate: methanol 10:0, 8:2, 7:3, 6:4, 4: 6, 3:7, 2: 8 and 0:10. The TLC analysis with the above solvent systems showed that hexane and ethyl acetate in a ratio of 7:3 gave the most pronounced separation with distinct spots and selected as the best solvent system.

Column chromatographic fractionation of the crude leaf extract

Crude methanol leaf extract of *C. aurea* was further subjected to bioassay guided fractionation. Crude leaf extract was fractionated by means of column chromatography using silica gel 60 (0.063-0.2 mm mesh size). Column chromatographic elution of crude leaf extract was done with solvent systems of gradually increasing polarity using hexane, ethyl acetate and methanol. The following ratios of solvent combinations were sequentially used in the elution process Hexane: ethyl acetate 100:0, 80:20, 70:30, 60:40, 40: 60, 30:70 and 20: 80; ethyl acetate: methanol 100:0, 80:20, 70: 30, 60:40, 40: 60, 30:70, 20: 80 and 0:100. A measured volume of each solvent combination was collected gradually with a 10 ml syringe and sprayed uniformly 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 distortion of this layer would result in a non-uniform drain of the fractions (Ode et al. 2011). Column chromatographic eluents of crude methanol leaf extract of *C. aurea* 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 7:3 ratio as a mobile phase. Visualization and identification of spots that indicate constituents of each eluent was done using an Ultra Violet lamp at a wavelength of 254 nm. Finally, eluents having similar constituents were pooled and concentrated using rotary evaporator at 38–40 ^{0}C and placed in a deep freeze until used in mosquito larvicidal tests (Ode et al. 2011; Tomass et al. 2011).

Preparation of test and control solutions for chromatographic fractions

For the column chromatographic fraction which is not readily soluble in water, 200 mg fraction was dissolved in 2 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 which test concentrations of 50, 100, 150, 200, 250 and 300 ppm were prepared by adding the appropriate volume of dilution. 0.1 mL of Tween 80 was used as an emulsifier. A mixture of 2 mL of ethanol and 0.1 mL of Tween 80 were diluted to 100 mL distilled water in standard measuring flask to serve as a negative control solution for larvicidal bioassays of column chromatographic fraction (WHO 2005). Larvicidal bioassay was conducted based on the above procedures.

Data analysis

Data from all replications were pooled and mean percent mortalities after 24 h exposure of the late third instar larvae of *An. arabiensis* that were treated with crude leaf extract of the five plants and column chromatographic fractions were determined by analysis of variance (ANOVA) using SPSS version 20. Tukey HSD test was also used to separate significant means ($p < 0.05$) in larval mortalities among plant extracts with each solvent, between solvent for each plant extract and column chromatographic fractions at different concentrations. For crude as well as column chromatographic fractions of leaf extract of each plants, the LC_{50}, LC_{90} at 95% fiducial limits of upper confidence limit and lower confidence limit, and chi-square values were determined using dosage mortality probit regression analyses using SPSS program version 20 to determine their larvicidal efficacies.

Results

Larvicidal activity of crude leaf extracts of test plants

The mean percent mortalities of larvae are presented in Table 1. All the test plants demonstrated larvicidal activities against 3rd instar *An. arabiensis* larvae at the test concentration. The highest mortality was recorded for methanol extract of *C. aurea*. Methanol extract of *C. aurea* showed better

Table 1 Larvicidal activity of ethanol and methanol crude leaf extract of test plants against *An. arabiensis* at 300 ppm

% Mean mortality ± SE		
Plant species	Solvents	
	Ethanol	Methanol
A. annua	79.11 ± 1.11^{Aa}	68.00 ± 1.15^{Ba}
C. aurea	85.33 ± 1.33^{Aac}	100.00 ± 0.00^{Bb}
C. anisata	71.11 ± 2.08^{Ab}	72.88 ± 1.11^{Aa}
D. stramonium	91.55 ± 1.69^{Acd}	90.22 ± 0.96^{Ac}
R. communis	93.33 ± 2.10^{Ad}	86.67 ± 1.33^{Bc}
Negative Control	0.00 ± 0.00^{Ae}	0.00 ± 0.00^{Ad}

*Each value (% mean ± SE) represents mean value of nine replicates

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

performance with 100% larval mortality followed by ethanol and methanol extracts of *R. communis* and *D. stramonium* with mortality ranging between 86%- 93.33%. However, methanol extract of *A. annua* and ethanol and methanol extracts of *C. anisata* showed lesser performance (mortality 68-72.88%). There was no statistically significant ($p > 0.05$) difference in the larvicidal activity of ethanol and methanol leaf extract of *C. anisata*, and *D. stramonium*. However, there was significant ($p < 0.05$) difference in the percentage mortality of ethanol and methanol leaf extracts of *A. annua*, *C. aurea* and *R. communis*. Besides, there was no significant difference ($p > 0.05$) in the larvicidal activities between ethanol leaf extract of *A. annua* and *C. aurea*, and between ethanol leaf extract of *D. stramonium* and *R. communis*.

Ethanol leaf extract of *D. stramonium*, *R. communis* and methanol extract of *C. aurea* and *D. stramonium* were subjected for dose-response bioassay to determine the LC_{50} and LC_{90} and the results are presented in Table 2.

The LC_{50} was lowest for methanol extract of *C. aurea* followed by ethanol extract of *R. communis*, *D. stramonium* and the methanol extract of *D. stramonium*. The result clearly showed that percentage of mortality is directly proportional to the concentration of the extract.

Larvicidal activities of column chromatographic fractions of methanol leaf extract of *C. aurea*

Nine fractions were collected during column chromatographic fractionation of the crude extract. Fraction 7, 8, and 9 were pooled based on their thin layer chromatographic result and designated as F7. The larvicidal activity of each fraction was tested separately and mean percentage mortality after 24 h are presented in Table 3. The result of larvicidal activities of column chromatographic fractions of methanol leaf extracts *C. aurea* revealed that F1-F3 caused a significant mortality in all concentrations ($p < 0.05$) but for fraction F4 significant mortality ($p < 0.05$) was recorded at 150, 200, 250 and 300 ppm concentrations. On the other hand no larval mortalities were recorded for fractions F5-F7 in all concentrations. F1 caused 100% mortality at 250 ppm and 300 ppm concentrations while F2 and F3 achieved 98.66% and 100% mortality at 300 ppm, respectively.

However, there was no statistically significant ($p > 0.05$) difference of larval mortality among F1, F2, and F3 at 250 and 300 ppm concentrations. Besides no statistically significant ($p > 0.05$) difference of larval mortality was observed for F1 at 150, 200, 250 and 300 ppm concentrations.

The average larval mortality of fractions F1-F4 of *C. aurea* methanol leaf extract after 24 h exposure were subjected to probit analysis for LC_{50} and LC_{90} determination and the results are presented in Table 4. The result revealed that F1 of *C. aurea* showed the lowest LC_{50} of 62.51 ppm and LC_{90} of 122.72 ppm followed by F3 with LC_{50} of 82.33 ppm and LC_{90} of 165.02 ppm. However, F2 and F4 showed higher LC_{50} of 100.37 ppm and 279.44 ppm, respectively.

Discussion

The result of the present study revealed that all the test plants caused high larval mortality ranging from 68%-100% after 24 h exposure at 300 ppm concentration. The larvicidal activities of the extracts vary among plant species and between solvents. This variation might have occurred due to difference in concentration and constituents of bioactive phytochemicals among the test plant species and the difference in the ability of solvents to extract the active ingredients. Ghosh et al. (2012) stated that the efficacy of phytochemicals against mosquito larvae can vary significantly depending on plant species, and extraction solvents. Similarly, Isah (2019) described that the type and concentration(s) of secondary molecule(s) produced by a plant are determined by the species, genotype, physiology and environmental factors during growth. Pandey and Tripathi (2014) in their review also indicated that the amount and type of phytochemicals extracted from plants are determined by the type of solvent and extraction methods. Crude methanol extract of *C. aurea* has a strong larvicidal activity with 100% mortality at 300 ppm and LC_{50} of 84.85 ppm. This potential biological activity could be attributing due to the presence of high amount of bioactive secondary metabolites. Phytochemical screening study conducted by Kemal et al. (2020) revealed that methanol leaf extract of *C. aurea* has phenolic compounds, tannin, alkaloids, flavonoids and saponin. To our knowledge, no study has been conducted on the larvicidal activities of *C. aurea* leaf extract against *An. arabiensis* larvae,

Table 2 LC_{50} and LC_{90} of leaf extracts from D. *stramonium*, *R. communis*, and *C. aurea* against *An. arabiensis* larvae

Solvent	Plant name	LC_{50} (ppm)	LCL	UCL	LC_{90} (ppm)	LCL	UCL	X^2 (df^b = 4)
Ethanol	*D. stramonium*	156.33	132.72	182.25	362.50	288.88	533.23	3.57
	R. communis	134.03	113.36	154.99	294.71	242.39	401.55	6.54
Methanol	*C. aurea*	84.85	67.51	100.54	192.29	159.44	253.69	2.46
	D. stramonium	167.68	147.06	188.68	308.60	262.14	404.21	1.33

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

however the plant is known for its larvicidal activities against Mediterranean fruit fly (*Ceratitis capitata*) and maize weevil (*Sitophilus zeamais* (Birhanu and Gutto 2015; Hiruy and Getu 2018). Besides, the finding of Korir et al. (2014) indicates that the plant has potential pesticidal activity against lice, maggot, and ticks. Ethanol leaf extract of *R. communis* and *D. stramonium* caused 93.33% and 91.55% larval mortality, respectively. These results are similar to the study conducted on the same plant extracts against *An. gambiae* in Eritrea (Okbatinsae and Haile 2017) though at a higher concentration, 1000 ppm. Similarly, Taha et al. (2011) evaluated ethanol extract of *R. communis* against *An. arabiensis* larvae and found the LC_{50} of 282.70 ppm which is a bit higher than the current finding. Differences in performance among the same plant species and extraction methods observed in the current study could have arisen due to a difference in environmental factors. According to Yang et al. (2018), 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. A study on ethanol leaf and seed extracts of *A. annua* (Ogbonna et al. 2010) was found to be lethal to larvae, pupa and adult females of *An. gambiae* as in the present study.

The current study revealed that column chromatographic fractions (F1, F2, F3) of methanol extract of *C. aurea* have a remarkable larvicidal activity against *An. arabiensis* larvae while no larval mortalities were observed for fractions F5, F6 and F7. The variation in toxicity of the different fractions suggested that the active compounds are confined to specific fractions based on their polarity. There was no previous study conducted on larvicidal activities of column chromatographic fractions of *C. aurea* leaf extract against *An. arabiensis*. However, the current result is similar with the finding of Tomass et al. (2011) on a different plant, (*Jatropha curcas*) where column chromatographic fractions (F1 and F2) of crude methanol *J. curcas* extract caused 100% mortality at 125 ppm against 3rd instar *An. arabiensis* larvae. Arivoli et al. (2016) also tested the larvicidal activity of nine fractions of *Sphaeranthus indicus* ethyl acetate whole plant extract against vector mosquitoes of *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* and his result revealed that amongst the fractions tested, fraction 6th showed 100% mortality against third

Table 3 Larvicidal activities of column chromatographic fractions of methanol leaf extract of *C. aurea* against *An. arabiensis*

Fractions	% Mortality ± SE					
	Concentrations (ppm)					
	50	100	150	200	250	300
F1	34.66 ± 2.66^{Aa}	80.00 ± 2.3^{Ba}	94.1 ± 1.33^{Ca}	98.66 ± 2.30^{Ca}	100.00 ± 0.00^{Ca}	100.00 ± 0.00^{Ca}
F2	21.33 ± 1.33^{Ab}	36.00 ± 2.30^{Bb}	74.66 ± 1.33^{Cb}	82.66 ± 1.33^{Cb}	93.33 ± 2.66^{Da}	98.66 ± 1.33^{Da}
F3	22.66 ± 1.33^{Ab}	54.66 ± 1.33^{Bc}	86.66 ± 3.52^{Cc}	96.00 ± 0.00^{Da}	98.66 ± 1.33^{Da}	100.00 ± 0.00^{Da}
F4	0.00 ± 0.00^{Ac}	2.66 ± 1.33^{Ad}	13.33 ± 1.33^{Bd}	30.66 ± 1.33^{Cc}	46.66 ± 2.66^{Db}	49.33 ± 1.33^{Db}
F5	0.00 ± 0.00^{Ac}	0.00 ± 0.00^{Ad}	0.00 ± 0.00^{Ae}	0.00 ± 0.00^{Ad}	0.00 ± 0.00^{Ac}	0.00 ± 0.00^{Ac}
F6	0.00 ± 0.00^{Ac}	0.00 ± 0.00^{Ad}	0.00 ± 0.00^{Ae}	0.00 ± 0.00^{Ad}	0.00 ± 0.00^{Ac}	0.00 ± 0.00^{Ac}
F7	0.00 ± 0.00^{Ac}	0.00 ± 0.00^{Ad}	0.00 ± 0.00^{Ae}	0.00 ± 0.00^{Ad}	0.00 ± 0.00^{Ac}	0.00 ± 0.00^{Ac}
Negative Control	0.00 ± 0.00^{Ac}	0.00 ± 0.00^{Ad}	0.00 ± 0.00^{Ae}	0.00 ± 0.00^{Ad}	0.00 ± 0.00^{Ac}	0.00 ± 0.00^{Ac}

*F1 = Fraction 1, F2 = Fraction 2, F3, Fraction 3, F4 = Fraction 4, F5 = Fraction 5, F6 = Fraction 6 and F7 = fraction 7 **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)

Table 4 LC_{50} and LC_{90} of column chromatographic fractions of *C. aurea* methanol leaf extract against *An. arabiensis* larvae

Fractions	LC50 (ppm)	LCL	UCL	LC90 (ppm)	LCL	UCL	X^2 (df[b] = 4
F1	62.51	48.24	74.73	122.72	102.33	161.02	0.20
F2	100.37	81.61	117.91	228.58	188.96	305.11	4.47
F3	82.33	67.03	96.40	165.02	139.18	210.82	1.67
F4	279.44	239.98	368.75	578.38	417.97	1264.39	0.71

*F1 = Fraction 1, F2 = Fraction 2, F3 = Fraction 3, F4 = Fraction 4

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

instar larvae of *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* at 100 ppm while other fractions resulted in less than 100% mortality. In the current study also F1-F3 caused the highest larval mortality than the rest. F1 and F3 were found more toxic to *An. arabiensis* larvae with LC_{50} of 62.51 ppm and 82.33 ppm (Table 4), respectively than the crude methanol extract with LC_{50} of 84.85 ppm (Table 2). The highest LC_{50} observed in crude methanol extract of *C. aurea* than its column chromatographic fraction F1 and F3 suggested that working with these fractions can reduce the lethal concentration to achieve the highest efficacy.

Conclusions

The current study revealed that crude ethanol and methanol leaf extracts of *A. annua, C. aurea, C. anisata, D. stramonium* and *R. communis* have potential larvicidal activities against 3rd instar larvae of *An. arabiensis*. Crude methanol extracts of *C. aurea*, crude ethanol leaf extract of *R. communis* and *D. stramonium* showed better larvicidal activities with more than 90% larval mortality after 24 h exposure. Moreover, column chromatographic fractions (F1and F3) of methanol extracts of *C. aurea* caused the highest larvicidal activities than the crude methanol extract of *C. aurea* with the lowest lethal concentrations. This implies that the active metabolites of F1 and F3 fractions of methanol extracts of C. *aurea* are promising for further botanical insecticide developments.

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

Authors' contributions AE conceived the study and wrote up the proposal. MH revised the proposal, supervised the study and revised the manuscript critically. AE carried the experiment, collected and interpreted the data and wrote the manuscript. EG supervised the study and revised the manuscript critically. ED participated in plant leaf extraction, column chromatographic fractionation of the extracts and write up of the manuscript. All authors approved the final version for submission.

Funding information Addis Ababa University, Addis Ababa, Ethiopia.

Data Availability The data sets used during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Conflict of interest Authors declare that they have no competing interest in this work.

Ethical approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

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