Larvicidal activity of *Clausena anisata* fruits and leaves extracts against *Anopheles gambiae* Giless.s, *Culex quinquefasciatus* Say and *Aedes egyptiae*

Clausena anisata meyve ve yaprak ekstraktlarının *Anopheles gambiae* Giles s.s, *Culex quinquefasciatus* Say ve Aedes egyptiae'ya karşı larvisidial aktivitesi

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ABSTRACT
Aim: The study aimed to evaluate larvicidal efficacy of *Clausena anisata* fruits and a leaf extracts against the 3rd instar *Anopheles gambiae* Giles s.s,*Culex quinquefasciatus* Say and *Aedes egyptiae* larvae.
Methods: The protocol developed by the World Health Organisation was adopted with minor modifications.
Results: The larvicidal activity study of *Clausena anisata* has revealed the selectivity of plant parts against the mosquito larvae. The leaf extracts displayed high potency against the 3rd instar *Anopheles gambiae* in which ethyl acetate leaf extract demonstrated high activity at LC_{50} of 0.0977 µg/mL followed by methanolic (LC_{50} 0.9362 µg/mL) and chloroform (LC_{50} 4.2384 µg/mL) extracts. The fruit ethyl acetate selectively exhibited the highest activity against *A. egyptiae* with LC_{50} of 5.3346 µg/mL. *Culex quinquefasciatus* larvae exhibited more resistance compared to *A. gambiae* and *A. egyptiae* larvae in which LC_{50} range of 78.658 – 178.895 µg/mL after 72 hours was displayed.
Conclusion: The leaves and fruits of *C. anisata* are potential source of botanical mosquito repellants and thus employed for the management of mosquito borne diseases. Leaves extracts selectively exhibited activity against *A. gambiae* indicating its potential use in the control of malaria. Fruits ethyl acetate extract had better activity against *A. egyptiae* larvae suggesting its use in the management of dengue fever, chikungunya and yellow fever viruses. It is apparently evident that *C. anisata* leaf extracts possess high potency against *A. gambiae* larvae compared to *Bacillus Thuringiensis*, subsp. Israeliensis (BTI®) which is the larvicidal product with high specificity against mosquito larva.
Keywords: Larvicidal; *Anopheles gambiae* Giles s.s; *Culex quinquefasciatus* Say;*Aedes egyptiae*; *Clausena anisata*.

ÖZET
Amaç: Çalışmanın amacı, *Clausena anisata* meyve ve yaprak ekstraktlarının 3. kabuk değiştirme safhasındaki *Anopheles gambiae* Giles s.s,*Culex quinquefasciatus* Say ve*Aedes egyptiae* larva türlerine karşı larvisidial etkinliğinin ortaya konulmasıdır.
Yöntemler: Dünya Sağlık Örgütünün geliştirdiği protokol minor değişikliklerlekullanıldı.
Bulgular:*Clausena anisata*'nın larvisidial aktivite çalışmaları bitki parçalarının sivirisinek larvalarına karşı selektif toksisitesini ortaya koydu.Yaprak ekstraktları, *Anopheles gambiae* sivrisineklerinin 3. evresine karşı yüksek etkinlik gösterdi. Yaprakların etil asetat ekstraktları LC_{50}=0.0977 µg/mL ile en yüksek aktiviteyi gösterirken, bunu metanolik (LC_{50}=0.9362 µg/mL) ve kloroform (LC_{50}=4.2384 µg/mL)ekstraktları takip etti. Meyve etil asetat ekstresi,LC_{50}=5.3346 µg/mL ile *A. egyptiae*'ya karşı selektif olarak en yüksek aktiviteyi gösterdi. *Culex quinquefasciatus* larvası 72 saat maruziyetle elde edilen 78.658–178.895 µg/mL LC_{50}değerleriyle *A. gambiae* and *A. egyptiae* larvalarıyla kıyaslandığında daha fazla direnç gösterdi.
Sonuç:*C. anisata*'nın yaprakları ve meyveleri bitkisel sivrisinek uzaklaştırıcılardır ve sivrisinek kaynaklı hastalıkların yönetiminde faydalanılabilir.Yaprak ekstraktlarının seçici olarak *A. gambiae*üzerine etkisi sıtma kontrolünde kullanılabileceğini göstermektedir. Meyvenin etil asetat ekstraktının *A. egyptiae* larvasına karşı daha iyi etkinlik göstermesi, dang humması, chikungunya vesarı humma virüslerine karşıda kullanılabilme fikrini ortaya koymaktadır. *C. anisata*yaprak ekstraktlarının *A. gambiae* larvasına karşı etkinliği sivrisinek larvasına karşı spesifik larvisidal etkinliği olan *Bacillus Thuringiensis*, subsp. Israeliensis (BTI®)'den daha yüksektir.
Anahtar kelimeler: Larvisidal; *Anopheles gambiae* Giles s.s; *Culex quinquefasciatus* Say;*Aedes egyptiae*; *Clausena anisata*.

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ReceivedAugust 10, 2015; **Accepted**November 11, 2015
DOI 10.5455/spatula.20151118060743
Published online in ScopeMed (www.scopemed.org).
Spatula DD..

INTRODUCTION

Mosquitoes are the oldest human enemy and today they are considered as a major public health threats because they are vectors responsible for transmission of various dreadful diseases that have significant threat to human health [1]. WHO has declared mosquitoes as public enemy number one due to the number of disease they transmit. Apart from their ability to harbour vector pathogens; they are also source of allergic reaction that includes local skin and systemic sensitivity [2]. Among many other insect, mosquito is considered as deadliest insect due to the role they play in the transmission of human dreadful diseases. For instance, genera *Anopheles*, *Aedes* and *Culex* are commonly known vector accountable for malaria, dengue fever, chikungunya, yellow fever, Rift Valley fever, filariasis, West Nile fever and Japanese Encephalitis [3].

Currently the incidence of mosquito borne diseases in Sub-Saharan Africa is high which has mainly attributed by the wide spread of mosquito in different parts of the world even the area where there were no history of mosquito [4]. Mosquito borne disease are major threat to over 2 billion of people in tropics [5]. Approximately 2 millions of people globally die and atleast 300 million cases of malaria each year [6]. About 1.2 billion of the global population is at risk of lymphatic filariasis and more than 120 million people are affected worldwide [7]. In 2010, there was an estimate of more than 200 million malaria cases and 660,000 malaria death globally in which 90% are in Sub-Sahara Africa with children under 5 years and pregnant women mostly affected [8]. In Tanzania, malaria is reported to be a most cause of infant, child and adult mortality with proximately two to three million new cases arising each year [9].

Dengue fever becomes the most rapidly spreading mosquito-borne viral disease and the estimates show that 2.5 billion people are at risk [10]. The dengue fever outbreak in 2014 left 5 death and 1,017 patients [11]. The prevalence of chikungunya has also been increasing over years. It was reported to increase from 15,783 to 59,535 in India between 2007 and 2014 respectively [12]. Several literature reports that people from tropical countries are at high risk and developing countries are mostly affected [13]. Due to the pathogenic diseases and serious harms caused by mosquitoes, vector control has been apprehended as the measure to control mosquito borne diseases [14].

The major tool in mosquito operation has been through the domestic insecticides interventions. The targets of adult mosquitoes has hindered by the fact that mosquitoes are highly mobile flying insects that can readily detect and avoid many intervention measures. The development of insecticides that targets mosquito eggs, larvae and pupae which are confined within relatively small aquatic habitats and cannot readily escape control measures has been viewed as an alternative approach of addressing mosquito borne diseases. The developments of insecticides that target larvae in the breeding habitat before they can mature into adult mosquitoes and disperse have attracted many scientists.

More than 1,005 plant species have been reported to possess insecticidal properties on which 297 have repellency activity and 27 are attractants that inhibit growth [5]. Plants have therefore viewed as potential source of usefully compounds for vector control. Unlike synthetic insecticides, bioinsecticides are biodegradable and thus are regarded as ecofriendly insecticides with low toxicity to natural opponents including humans and other mammals [15]. For instance clove oil, fennel oil, geranium oil catnip, citronella, lavender, neem, and black pepper and pyrethrum are commercially available insecticides [16]. Likewise, other biodiversity resources including wild and domestic plants have for many years proved to be a rich source of larvicidal agents [3]. For instance, quasin from quassia tree is a good mosquito larvicide as it is over five times as active as carbaryl, a synthetic antilarval agent [17]. Searching for effective larvicides from plants used for the management of insect vectors including mosquitoes offers a promising source. The aim of this study therefore was to evaluate larvicidal efficacy of *Clausena anisata* fruits and leaf extracts against *Anopheles gambiae*Giless.s, *Culex quinquefasciatus* Sayand *Aedes egypti*.

METHODS AND MATERIAL

Chemicals and organisms tested

Chloroform, ethyl acetate, methanol and dimethyl sulfoxide (DMSO) was purchased from Avantor performance materials India l. *Anopheles gambiae* Giles s.s, *Culex quinquefasciatus* Say and *Aedes egyptiae* larvae were obtained and reared at the Tropical Pesticides Research Institute (TPRI) Arusha, Tanzania.

Collection and preparation of plant material

Fruits and leaves of *Clausena anisata* were collected from Monduli in Arusha region, Tanzania on 26th January 2015. Plant species was identified by a Mr. Suleiman Haji, a botanist from the department of Botany, University of Dar es Salaam, and the voucher specimen coded CA 14 is kept at Nelson

Mandela African Institute of Science and Technology, Arusha.

Preparation of extracts

The fresh fruits and dried leaves of *C. anisata* were pulverized using the heavy duty laboratory blender. The pulverized materials (800 g each plant part) were sequentially macerated using chloroform, ethyl acetate and methanol for 48h twice for each solvent. The respective extracts were filtered through Whatman filter paper number 1 in a glass column. The solvents were removed through the vacuum using a rotary evaporator to obtain 240g, 200g, 160g of *C. anisata* fruits methanolic, ethyl acetate and chloroform extracts respectively. From *C. anisata* leaves, 216g, 136g and 120g of methanolic, ethyl acetate and chloroform extracts were obtained respectively. All extracts were stored in refrigerator at -20°C until the testing time.

Larvicidal activity Assay

The bioassay tests were performed according to World Health Organization (WHO) protocol [18] with minor modification. According to the protocol, mosquito larvae were fed with tetramine ® for *A gambiae* and dog biscuits for *C. quinquefasciatus* and *A. aegypti* during the whole time of experiment. Stock solutions (100 mg/mL) of each plant extracts were prepared by dissolving 100 mg in 1 mL of DMSO. Different levels of concentration 50, 100, 200, 400, and 800µg/mL from the stock solution were prepared by serial dilution. Each of these desired concentrations added to distilled water to make100 ml each, in plastic cups. Ten late third instars mosquito larvae were introduced in the test solution and mortality were observed after 24h, 48h and 72h. The plastic cups with mosquito larvae, distilled water and DMSO (0.5%) were considered as negative control tests. All tests were carried out in triplicate for statistically significant results, under controlled temperature (25 ± 2 °C) and relative humidity of 75-85%. Dead larvae were identified when they are immobile and unable to reach the water surface when probed by the dropper .The mean results of the percentage mortality were plotted against the logarithms of concentrations using the Fig P computer program (BiosoftInc, USA). The concentrations killing fifty percent of the larvae (LC_{50}), confidence interval (C.I) and regression coefficient (R^2) were calculated from the regression equations obtained from the graphs**.**

RESULTS

The strong odor of *Clausena anisata* leaves and fruits has been associated with mosquito killing properties by the remote rural communities in Tanzania. Consequently, the leaves of *C. anisata* plant have for many years utilized for the management of malaria vectors (*A. gambiae*) in Tanzania. The plant is likewise used in some parts of Philippines [19]. In order to validate this use, fruits and leaf extracts were evaluated for larvicidal activity against *Anopheles gambiae*, *Culex quinquefasciatus* and *Aedes egyptiae* larvae. Mosquito larvae were exposed to extracts prepared in dimethyl sulphoxide at concentration range of 50 – 800 µg/mL and mortality was recorded after 24, 48 and 72h of exposure. According to [20, 21], larvicidal activities of the plant extracts is considered as inactive when the LC_{50} is greater than 750 µg/mL, weakly effective (LC_{50} 200 - 750 µg/mL), moderate (LC_{50} 100 - 200 µg/mL), effective (LC_{50} 50 -100 µg/mL), and highly effective (LC_{50} less than 50 µg/mL). The tested plant extracts exhibited different level of larvicidal activities. Fruits extracts exhibited larvicidal activities with the LC_{50} range of 5.3346 - 145.4839 µg/mL, 1.9809 - 132.609 µg/mL and 78.658 - 293.405 µg/mL (Table 1, 2 and 3) while the leaf extracts exhibited larvicidal activities with LC50 range of 0.0977-140.2901 µg/mL, 111.825- 8,468.02 µg/mL and 5.3346-145.4839 µg/mL (Table 4,5 and 6) against *Aedes egyptiae*, *Anopheles gambiae* and *Culex quinquefasciatus* larvae respectively. The order of mosquito larvae sensitivity to *C. anisata* extracts are in the order of *Anopheles gambiae*>*Aedes egyptiae*>*Culex quinquefasciatus*. It was evident that *C. quinquefasciatus* exhibited more resistance compared to *Anopheles gambiae* by the factor of 39 as elaborated by results obtained from the most active *C. anisata* extracts against *C. quinquefasciatus* (LC_{50} 78.658 µg/mL) and *Anopheles gambiae* (LC_{50} 1.9809 µg/mL) (Table 3).

As depicted in (Table 1, 2 and 3), fruits extracts exhibited larvicidal activity against the three mosquito larvae. The activity was species specificity, which clearly revealed that fruit ethyl acetate extract had higher larvicidal activity with LC_{50} 5.3346 µg/mL against *Aedes egypti*ae; fruit methanolic extract exhibited the highest activity with LC_{50} 1.989 µg/mL against *Anopheles gambiae* and fruit chloroform extract exhibited high activity with LC_{50}78.658 µg/mL against *Culex quinquefasciatus* in 72h of exposure.

Table1. Larvicidal activity of *Clausena anisata* fruits extracts against *Aedes aegypti*

Extracts Code	Time of observation	LC_{50} (µg/mL)	95 % (UCL – LCL)	R^2	Regression equation
CAFC	24h	122.2383	88.0222-169.7535	0.9476	y=66.038logx-7.835
	48h	64.0934	44.3640-92.5957	0.9891	y=65.9logx - 69.069
	72h	77.4349	50.6926-118.2841	0.917	y=51.187logx- 6.689
CAFE	24h	70.009	46.4497-105.5186	0.9595	y=52.858logx- 7.531
	48h	21.5086	12.2243-37.8442	0.8939	y=42.91logx- 7.1824
	72h	5.3346	2.2726-12.5219	0.9275	y=32.811logx+ 26.143
CAFM	24h	145.4839	104.735-202.0844	0.8833	y=65.989logx- 92.722
	48h	82.3302	53.1369-127.5622	0.9105	y=49.526logx- 4.87
	72h	71.1235	40.2051-125.8181	0.9267	y = 38.017logx - 20.408
CONTROL	**NM**	-	-	-	-

CAFC – *Clausena anisata* fruits chloroform extract, CAFE – *Clausena anisata* fruits ethyl acetate extract, CAFM- *Clausena anisata* fruits methanol extracts, NM- No mortality at all levels of concentration tested, UCL - Upper Confidence limit, LCL - Lower Confidence limit, LC_{50}- Lethal concentration (concentration to kill 50% of test organisms), CI-Confidence Interval, R^2-Regression coefficient

Table 2. Larvicidal activity of *Clausena anisata* fruits extracts against *Anopheles gambiae*

Extracts Code	Time of observation	LC_{50} (µg/mL)	95% (UCL – LCL)	R^2	Regression equation
CAFC	24h	92.5632	66.6994-128.4555	0.9048	y=66.117logx-80.015
	48h	60.6641	39.0667-94.20103	0.967	y=63.616logx- 63.423
	72h	43.1228	29.4793-63.0806	0.9947	y=73.604logx- 70.321
CAFE	24h	132.609	100.8693-174.3357	0.9189	y=79.267logx- 118.25
	48h	69.8011	52.4486-92.8913	0.9185	y=97.952logx- 130.61
	72h	34.4056	21.4056-55.2977	0.9489	y=51.093logx- 28.511
CAFM	24h	38.932	26.6365-56.9030	0.9674	y=73.765logx- 67.309
	48h	40.9725	31.4112-53.4440	1	y=129.03logx- 158.06
	72h	1.9809	0.6321-6.2082	0.9611	y=24.508logx+ 42.724
CONTROL	**NM**	-	-	-	-

CAFC – *Clausena anisata* fruits chloroform extract, CAFE – *Clausena anisata* fruits ethyl acetate extract, CAFM- *Clausena anisata* fruits methanol extracts, NM- No mortality at all levels of concentration tested, UCL - Upper Confidence limit, LCL - Lower Confidence limit, LC_{50}- Lethal concentration (concentration to kill 50% of test organisms), CI-Confidence Interval, R2-Regression coefficient

Table 3. Larvicidal activity of *Clausena anisata* fruit extracts against *Culex quinquefasciatus*

Extracts Code	Time of observation	LC_{50} (µg/mL)	95 %(UCL – LCL)	R^2	Regression equation
CAFC	24h	150.693	103.783-218.81	0.9066	y=57.812logx-75.92
	48h	79.807	52.958-120.267	0.9785	y=52.878logx-0.576
	72h	78.658	52.847-117.0748	0.9634	y=54.529logx-3.373
CAFE	24h	162.465	123.557-213.6344	0.9382	y=79.199logx-125.1
	48h	102.074	75.6008-137.817	0.9344	y=80.755logx- 112.23
	72h	80.713	57.780-112.754	0.9607	y=72.524logx- 88.299
CLFM	24h	293.405	203.711-422.60	0.9123	y=59.433logx-96.649
	48h	261.445	189.7511-360.2267	0.9809	y = 67.66logx - 113.56
	72h	178.895	127.746-250.536	0.9432	y=64.387logx- 95.038
CONTROL	**NM**	-	-	-	-

CAFC – *Clausena anisata* fruits chloroform extract, CAFE – *Clausena anisata* fruits ethyl acetate extract, CAFM- *Clausena anisata* fruits methanol extracts, NM- No mortality at all levels of concentration tested, UCL - Upper Confidence limit, LCL - Lower Confidence limit, LC_{50}- Lethal concentration (concentration to kill 50% of test organisms), CI-Confidence Interval, R2-Regression coefficient

For the leaf extracts (Table 4, 5 and 6), leaf ethyl acetate extract demonstrated larvicidal activity with LC_{50} 42.2848 µg/mL, 148.9425 µg/mL and 199.398 µg/mL against *Anopheles gambiae, Aedes egypti*ae and *Culex quinquefasciatus* respectively after 24 h of exposure. The highest activity was identified after 72h with LC_{50} 0.0977 µg/mL, 40.4955 µg/mL and 111.825 µg/mL against *Anopheles gambiae, Aedes egypti*ae and *Culex quinquefasciatus* respectively. The leaf chloroform and methanolic extracts had moderate larvicidal activities as summarized in Table 6.

Table 4. Larvicidal activity of *Clausena anisata* leaf extracts against *Aedes aegypti*

Extracts Code	Time of observation	LC50 µg/mL	95% (UCL – LCL)	R^2	Regression equation
CALC	24h	333.9405	271.7345-410.386	0.961	y = 105.2logx - 215.49
	48h	287.0467	234.2441-351.7519	0.9533	y = 119.27logx - 243.16
	72h	236.7697	189.7234-295.4819	0.9438	y = 109.45logx - 209.87
CALE	24h	148.9425	110.1831-201.3363	0.9301	y = 71.946logx - 106.34
	48h	81.1188	35.4883-185.4203	0.9518	y = 94.698logx - 130.79
	72h	40.4955	25.4472-65.1734	0.8958	y = 58.832logx - 44.567
CALM	24h	828.8481	511.5216-1343.024	0.8677	y = 44.931logx - 81.13
	48h	402.8643	274.498-591.2557	0.9168	y = 56.524logx - 97.254
	72h	279.7041	175.9296-444.6911	0.8893	y = 46.772logx - 64.437
CONTROL	**NM**	-	-	-	-

CALC – *Clausena anisata* leaves chloroform extract, CALE – *Clausena anisata* leaves ethyl acetate extract, CALM- *Clausena anisata* leaves methanol extracts, NM- No mortality at all levels of concentration tested, UCL - Upper Confidence limit, LCL - Lower Confidence limit, LC_{50}- Lethal concentration (concentration to kill 50% of test organisms), CI- Confidence Interval, R2-Regression coefficient

Table 5. Larvicidal activity of *Clausena anisata* leaf extracts against *Anopheles gambiae*

Extracts Code	Time of observation	LC_{50} (µg/mL)	95% (UCL – LCL)	R^2	Regression equation
CALC	24h	140.2901	103.5561-190.0545	0.8855	y = 71.429logx - 103.36
	48h	35.9073	21.3412-60.4151	0.8984	y = 46.599logx - 22.47
	72h	4.2384	1.5856-11.3294	0.9937	y = 28.474logx + 32.141
CALE	24h	42.2848	28.3067-63.1653	0.9087	y = 69.759logx - 63.441
	48h	3.1259	1.11367-8.7737	1	y = 33.223logx + 33.555
	72h	0.0977	0.0124-0.7696	1	y = 16.611logx + 66.777
CALM	24h	28.836	16.1243-51.5685	0.9256	y = 42.282logx - 11.729
	48h	3.4044	1.2423-9.3290	0.8753	y = 27.772logx + 35.224
	72h	0.9362	0.2472-3.5445	0.9292	y = 21.029logx + 50.602
CONTROL	**NM**	-	-	-	-

CALC - *Clausena anisata* leaves chloroform extract, CALE -*Clausena anisata* leaves ethyl acetate extract, CALM-*Clausena anisata* leaves methanol extracts, NM- No mortality at all levels of concentration tested, UCL - Upper Confidence limit, LCL - Lower Confidence limit, LC_{50} - Lethal concentration (concentration to kill 50% of test organisms), CI-Confidence Interval, R2-Regression coefficient

Table 6. Larvicidal activity of *Clausena anisata* leaf extracts against *Culex quinquefasciatus*

Extracts Code	Time of observation	LC50 μg/mL	95% (UCL – LCL)	R2	Regression equation
CALC	24h	471.639	347.2840-640.5225	0.9612	y = 79.215logx - 161.79
	48h	333.711	266.2742-418.2266	0.9655	y = 107.4logx - 221.01
	72h	225.0363	171.7814-294.8009	0.9956	y = 80.306logx - 138.9
CALE	24h	199.398	149.2626-266.3729	0.9385	y = 74.883logx - 122.21
	48h	170.1638	124.9633-231.7135	0.8659	y = 78.53logx - 125.19
	72h	111.825	77.643-161.0503	0.8977	y = 66.462logx - 86.15
CALM	24h	8,468.02	3465.826-20689.828	0.9237	y = 27.14logx - 56.6
	48h	2,633.12	1391.3169-4983.1796	0.8906	y = 38.007logx - 80.018
	72h	151.3363	92.2255-248.3322	0.8993	y = 43.786logx - 45.451
CONTROL	**NM**	-	-	-	-

CALC - *Clausena anisata* leaves chloroform extract, CALE - *Clausena anisata* leaves ethyl acetate extract, CALM- *Clausena anisata* leaves methanol extracts, NM- No mortality at all levels of concentration tested, UCL - Upper Confidence limit, LCL - Lower Confidence limit, LC_{50}- Lethal concentration (concentration to kill 50% of test organisms), CI- Confidence Interval, R2-Regression coefficient

DISCUSSION

Clausena anisata has been exploited in East Africa and some Asian countries for the management of mosquito borne diseases for many years [19]. This use prompted many scientists to validate the mosquito killing properties of the leaves of *C. anisata*. Previous study revealed that the *C. anisata* growing in South Africa acetone and dichloromethane extracts did not exhibit larvicidal activity against *Aedes aegypti* when evaluated at the concentration range of 40 - 120 ppm [22]. Only hexane extract induced mortality of *Aedes aegypti* larvae with LC_{50} values of 68.30 and 59.65 ppm after 24 h and 48 h respectively [15]. It is however interesting to observe that the current study revealed that chloroform and ethyl acetate leaf extracts from *C. anisata* growing in Tanzania had larvicidal against *Aedes aegypti* larvae with LC_{50} values of 236.7697and 40.4955 μg/mL after 72 h respectively. Chloroform and ethyl acetate are relatively comparable in polarity with dichloromethane and acetone respectively which suggests that the difference in larvicidal activity between the South Africa and Tanzania *Clausena anisata* leaf extracts is primarily on the chemical profiles.

The essential oil from the leaves of *C. anisata* growing in India was also reported to exhibit the larvicidal activity with LC_{50} values of 140.96, 130.19 and 119.59 ppm against *Anopheles gambiae*, *Culex quinquefasciatus* and *Aedes egyptiae larvae* respectively after 24 h [23]. The chemical profile of the essential oil from *C. anisata* growing in India was established to contain β-pinene, sabinene, germacrene, estragle and linalool [23]. Likewise, pyranocoumarin (seselin) was established to be the main ingredient in the South African *C. anisata* leaf hexane extracts [22]. These compounds were further established to exhibit larvicidal activities as reported in [22].

The growing evidence on the scientific validation on the larvicidal activity of the leaves of *C. anisata* proves that this plant is indeed a reservoir of potent mosquito larvicides. It is however apparent that the larvicidal activity of *C. anisata* fruit extracts are reported for the first time in this report. In general, larvicidal effect described by *C. anisata* fruits and leaf extracts justify its traditional use in managing mosquito. Therefore mosquito borne disease can be controlled by using mosquito repellent or killing agents derived from this plant. However, targeting *C. quinquefasciatus*, *A. aegyti* and *A. gambiae* at the larvae stage provides the best option for the management of mosquito borne diseases and thus Sub-Saharan Africa should seriously considered and prioritize for development, evaluation and implementation of botanical larvicides.

CONCLUSION

Threat of mosquito borne diseases especially in developing countries can be minimized by vector control. The use of environmentally safe, biodegradable and cheaply available indigenous methods is most recommended approach. Therefore the compounds with mosquito repellency properties from wild and domestic plants traditionally used for management of mosquito offers a promising source. The current study publicizes *C. anisata* leafs ethyl acetate extract to have high larvicidal activity as compared to other extracts against *A. gambiae*, *C. quinquefasciatus* and *A. aegytiae*. On other side *C. anisata* fruit chloroform, ethyl acetate and methanolic extracts exhibited high larvicidal activity which is species specific. Thus, these extracts are potential source of mosquito larvicides that can be included in mosquito intervention programs.

ACKNOWLEDGEMENT

The authors are grateful to the government of Tanzania through Nelson Mandela African Institution of science and Technology and UNDP through small grants programme: Project number TAN/SGP/OP5/Y3/STAR/BD/13/10 for funding the study. Mr. Suleiman Haji, a botanist from the Department of Botany, University of Dar es Salaamis thanked for plant identification.

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