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Larvicidal and phytochemical properties of *Callistemon rigidus* R. Br. (Myrtaceae) leaf solvent extracts against three vector mosquitoes

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ABSTRACT

Background & objectives: Due to ever-growing insecticide resistance in mosquito vectors and environmental contamination by synthetic insecticides, plants may be a source of alternative agents for mosquito control. Therefore, the present investigation involved the determination of larvicidal and phytochemical properties of *Callistemon rigidus* leaf extracts against *Anopheles gambiae*, *Aedes aegypti* and *Culex quinquefasciatus*.

Methods: The standard protocol of WHO was used for larval tests. Twenty five IV instar larvae were exposed to various concentrations from 125–1000 ppm for methanol crude extract (MCE), hexane (HF), chloroform (CF), ethyl acetate (EAF) and methanol (MF) fractions, from 250–2000 ppm for aqueous extract (AE) and 2500 ppm for Diclorvos. The mortality was observed 24 h post-exposure. The LC_{50} and LC_{90} values were determined by Probit analysis.

Results: The phytochemical analysis revealed that the presence of alkaloids, steroids, saponins, terpenoids, tannins and phenolic compounds, lipids, fats and fixed oils in MCE; terpenoids, steroids, lipids, fats and fixed oils in HF; terpenoids in CF; tannins and phenolic compounds in EAF and alkaloids, tannins, saponins and phenolic compounds in MF. Against *Ae. aegypti*, HF was the most active fraction with LC_{50} of 56.25 ppm. Against *An. gambiae*, HF demonstrated its potential mosquito larvicide killing relatively all exposed larvae at all concentrations with LC_{50} of 17.11 ppm. Against *Cx. quinquefasciatus*, only MCE and HF exhibited larvicidal activity with LC_{50} of 447.38 and 721.95 ppm, respectively.

Interpretation & conclusion: *Callistemon rigidus* exhibited some promising larvicidal activity against medically important vector mosquitoes. Studies are indicated to identify the active compounds from this plant for developing mosquito larvicides

Key words *Callistemon rigidus*, fractions, larvicide, mosquito vectors, phytochemicals.

INTRODUCTION

In tropical countries, >2 billion people are at risk from mosquito-borne diseases such as malaria, dengue, dengue haemorrhagic fever, Japanese encephalitis and filariasis[1]. These infectious diseases mainly impact the poor people of the tropical countries. *Culex*, *Aedes* and *Anopheles* (Diptera: Culicidae) mosquitoes are the most important vectors involved in diseases transmission to humans[2]. The responsible pathogens are transmitted by bites of blood sucking mosquitoes which are considered to be harmful towards the populations in tropical and subtropical regions[3]. An estimated 50 million people are infected with dengue each year caused by *Ae. aegypti*[1]. Malaria, caused by *An. gambiae*, has a crippling effect on Africa's economic growth and perpetuates vicious cycles of poverty[4]. The Malaria Situation Room provides strategic support to 10 high burden countries in Africa, namely Burkina Faso, Cameroon, Côte d'Ivoire, the Democratic Republic of the Congo, Ghana, Mozambique, Niger, Nigeria, Uganda and the United Republic of Tanzania. These 10 countries are estimated to account for more than 3,89,000 malaria deaths each year, representing about 60% of all malaria deaths in Africa in 2012[4]. The worst is that the cardiac involvement has been noticed in malaria, making it an overlooked important complication[5]. *Culex quinquefasciatus* is a vector of important diseases, such as West Nile virus, filariasis, Japanese encephalitis, St. Louis encephalitis, and bancroftian filariasis (*Wuchereria bancrofti*)[6].

One of the approaches to control these mosquito-borne diseases and deaths is the interruption of the disease transmission, by killing or preventing mosquito larvae from maturing into biting adults that cause diseases[2]. Conventional mosquito larvicides such as temephos, S-methoprene, *etc*. are often used[7]. Repeated use of these synthetic insecticides for mosquito control has resulted in development of resistance, undesirable effects on non-target organisms, fostered environmental and human health concern[8]. Search for an alternative in the develop-

ment of environmentally safe, biodegradable insecticides for mosquito control is ongoing over the world towards plant products[9]. Phytochemicals derived from plant resources acting as larvicides have been observed by different researchers[8–11]. Natural products of plant origin are safer to use than the synthetic insecticides. Therefore, biological and eco-friendly natural resources are broad search area for the control of vectors of medical importance[11].

Callistemon is a genus of 34 species of shrubs in the family of Myrtaceae, all of which are endemic to Australia. It is sometimes considered as synonym of *Melaleuca*[12]. *Callistemon* can be propagated either by cuttings (some species more easily than others), or from the rounded seeds. Flowering is normally in spring and early summer (October–December), but conditions may cause flowering at other times of the year[12]. *Callistemon rigidus* is a stiff and upright shrub characterized by red flower spikes that are shaped like bottle brushes. Flowers are comprised of red, showy stamens each approximately 1 inch long[13]. This genus has been found to be rich in phytochemicals such as triterpenoids, flavonoids, steroids and saponins[12]. In Cameroon, *C. rigidus* is known in folk medicine for its anticough, antibronchitis effects and its essential oils exhibited antifungal activity against *Phaeoramularia angolensis*[14] and *Aspergillus flavus*[15]. Essential oil extracted from its leaf has been found to be effective against IV instar larvae and early pupae of *Ae. aegypti*, *An. gambiae* and *Cx. quinquefasciatus*[2].

Mosquitoes in the larval stage are striking targets for pesticides because they rear in water and therefore, very easy to handle them in this atmosphere[16]. The present study was carried out to report the phytochemical composition and determine the larvicidal activity of *C. rigidus* leaf solvent extracts against three important vectors, viz. *An. gambiae*, *Ae. aegypti* and *Cx. quinquefasciatus*. Throughout our online search, this was the first report of toxicity of different solvent extracts from *C. rigidus* leaf against those important human vector mosquitoes.

MATERIAL & METHODS

Collection of plant material

Fresh leaves of *C. rigidus* were collected in October 2011 (0600–1100 hrs GMT) in Ngaoundere (latitude 7° 22′ North and longitude 13°34′ East, altitude of 1100 masl), located in the Adamawa region (plateau), Cameroon. These plants were identified for confirmation at the National Herbarium of Cameroon, where the voucher specimen number of 18564/SRF/CAM was deposited. Leaves were dried at room temperature of 25 ± 3°C and 81 ± 2% RH, and ground in powder using electric grinder until the powder passed through a 0.4 mm mesh sieve. The powder was stored in opaque containers inside a refrigerator at – 4°C and transported by road in February 2012 to the Faculty of Pharmaceutical Sciences, Agulu; Nnamdi Azikiwe University, Awka; Anambra state, Nigeria where the experiments were carried out and then stored in a freezer at – 4°C until needed.

Extraction and fractionation of plant material

The extraction scheme was performed according to the method adopted by Okoye and Osadede[17]. From the collection of plant material powder, 700 g were extracted for three days by cold maceration in methanol shaking it thrice per day (morning, noon and afternoon). The maceration process was then repeated thrice for maximal extraction. The methanol crude extract was then collected and concentrated almost to dryness under vacuum at 40°C using rotary evaporator RE300 (ROTAFLO, England). The methanol crude extract was first absorbed on silica gel (60–200 mesh size) and sequentially extracted using hexane, chloroform, ethyl acetate and methanol in increasing order of polarity. All the fractions so obtained were filtered many times adding fresh solvent until clear phase was obtained before passing to the next solvent using Whatman No. 1 filter paper (Size: 24 cm, England). The yield of the extract and fractions was 27.28, 8.18, 11.35, 12.42 and 37.62% for methanol crude extract (MCE), hexane (HF), chloroform (CF), ethyl acetate (EAF) and methanol (MF) fractions respectively. The same rotary evaporator was used to concentrate the fractions at 40±5°C. For the aqueous extraction, 200 g of ground botanical was soaked in 1 L distilled water for 6 h with occasional shaking to dissolve the active components. The suspension was later filtered using a fine muslin cloth. The filtrate was then lyophilized (freeze-dried) to remove the water solvent using the Yorco Freeze-drying machine (Lyophlizer); manufactured by York Scientific Industries Pvt. Ltd. (India). The aqueous extract (AE=11.62%) was obtained. Extracts and fractions were stored in a freezer at – 4°C.

Test organisms

The larvae of *Ae. aegypti*, *An. gambiae* and *Cx. quinquefasciatus* were from the colonies reared in the insectarium of the Faculty of Pharmaceutical Sciences, Agulu; Nnamdi Azikiwe University, Awka; Anambra state, Nigeria. *Aedes aegypti* and *Cx. quinquefasciatus* were formerly collected from WHO/National Arbovirus and Vector Research Centre, Enugu, Enugu state, Nigeria and *An. gambiae*, from Awka market, Anambra state,

Nigeria inside the gutter and identified by WHO/National Arbovirus and Vector Research Centre, Enugu, Enugu state, Nigeria. The larvae of *Ae. aegypti* and *Cx. quinquefasciatus* were fed with chicken feed (grower) mixed with fish feed in 3:1 ratio. Ground chicken feed (grower), yeast and fish feed in 3:1:2 ratios were floated on the water surface for *An. gambiae* feeding. On every alternate day, the water from the culture bowl was changed carefully until the larvae turned into the IV instar used for the bioassay. They were maintained at 28 ± 4°C, 81 ± 5% RH and under 12L: 12D photoperiod cycles. The adults were fed with 10% sugar solution. As from four days after emergence, the females of *Ae. aegypti* were offered a Guinea pig (*Cavia porcellus*) and one month chicken was used for blood feeding of *An. gambiae* and *Cx. quinquefasciatus*. This study was given an ethical approval from Anambra State University Teaching Hospital, Amaku, Awka; Anambra state, Nigeria Ethics Review Committee with the reference number of ANSUTH/AA/ECC/36.

Larvicidal bioassays

The WHO standard procedure[18] was followed to determine the toxicity of the plant extracts and fractions against *Ae. aegypti, Cx. quinquefasciatus* and *An. gambiae* IV instar larvae. The stock solutions of the extracts and fractions were performed using Tween 80 as emulsifier. The stock solutions were further diluted up to 100 ml by adding tap water. From these stocks, various concentrations from 125 to 1000 ppm were made and 1 ml of Tween-80 dropped in 99 ml of tap water was used as negative control. These controls were set up for each replicate, mosquito species and extract or fraction. For aqueous extract, 2 ml of distilled water was used for dissolution and made up to 100 ml as stock solution by adding tap water. From the stock solution, concentrations ranging from 250 to 2000 ppm were made. For comparison, a commercial formulation of Daksh Insecticide (Dichlorvos 100% E.C. w/v) (2500 ppm, recommended concentration), bought from Awka market (Anambra state, Nigeria), was used as positive control and 1 ml of distilled water in 99 ml of tap water was used as negative control. All the concentrations were chosen after a preliminary test for all extracts and fractions. Twenty-five IV instar larvae of each mosquito species were released into each 250 ml beaker containing 100 ml of aliquots and mortality was recorded after 24 h post-exposure. No food was provided either to the tests or controls during the experiments. The dead larvae were expressed as percentage mortality at each concentration. In cases where bioassay tests showed more than 20% of mortality in negative control, these were discarded and repeated. However, when (negative) control mortality ranged from 5–20%, the observed percentage mortality was corrected by Abbott's formula[19]. The larvae were considered as dead, if they were not responsive to a gentle prodding with a fine needle. All bioassays were carried out at room temperature of 27 ± 3°C and 78 ± 4% of relative humidity. Experiments were set in four replicates along with control.

Phytochemical tests

The phytochemical screening of *C. rigidus* leaf extract and fractions was carried out following the standard protocols[20–21].

Statistical analysis

The corrected mortality was determined using Abbott's[19] formula whenever required. The mortality data were subjected to ANOVA procedure using SPSS 17.0. Duncan test ($p = 0.05$) was applied for mean separation. Probit analysis[22] was applied to determine lethal dosages causing 50% (LC_{50}) and 90% (LC_{90}) mortality of larvae 24 h post-exposure.

RESULTS

The investigation results from qualitative phytochemical composition of *C. rigidus* presented in Table 1 re-

Table 1. Phytochemical compounds of *C. rigidus* leaf extract and fractions

Phytochemical compounds	Extract and fractions				
	Methanol crude extract	Hexane fraction	Chloroform fraction	Ethyl acetate fraction	Methanol fraction
Alkaloids	+++	–	–	–	+
Tannins & Phenolic compounds	+++	–	–	+++	++
Steroids	++	+	–	–	–
Saponins	+++	–	–	–	+++
Lipids, fats and fixed oils	++	++	–	–	–
Terpenoids	++	++	+	–	–

(+): Present at low concentration; (++): Present at moderate concentration; (+++): Present at high concentration; (–): Not present.

vealed the presence of alkaloids, tannins and phenolic compounds, steroids, saponins, lipids, fats and fixed oils and terpenoids in MCE; steroids, terpenoids and lipids, fats and fixed oils in HF; terpenoids in CF; tannins and phenolic compounds in EAF and alkaloids, tannins, saponins and phenolic compounds in MF.

The larvicidal toxicity of the organic extracts and fractions of *C. rigidus* was found to be mosquito species dependent and extracts or fractions dependent. The results revealed that different plant fractions varied significantly in their larvicidal potentials.

The results of extract and fractions against IV instar larvae of *Ae. aegypti* 24 h post-exposure are presented in Table 2. HF achieved maximum mortality of 100% at 1000 and 500 ppm; 96% at 250 ppm and 81.33% at 125 ppm with LC_{50} value of 56.25 ppm. This achievement made it the most active of all extract and fractions tested. Statistically, there was just a little difference among concentrations (F= 25.28; $p<0.05$). Chloroform fractions achieved 81.33% of mortality in the highest concentration (1000 ppm) and 5.67% at the lowest concentration (125 ppm) with LC_{50} value of 504.10 ppm. The highest concentration exhibited a mortality rate of 45.33, 25.33 and 10.67% for MCE, MF and EAF, respectively. The LC_{50} values were 1024.88, 3634.62 and 8765.97 ppm for MCE, MF and EAF, respectively. The aqueous extract recorded good results as well. It attained larvae dead percentage of 26.67, 81.33 and 85.33% at 500, 1000 and 2000 ppm, respectively with LC_{50} value of 761.26 ppm (Table 3).

The results of extracts and fractions against IV instar larvae of *An. gambiae* 24 h after treatment are summarised in Table 4. HF still demonstrated its potential mosquito

Table 2. Larvicidal activity of *C. rigidus* extract and fractions against IV instar larvae of *Aedes aegypti* 24 h post-treatment

Extract and fractions	Conc. (ppm)	% mortality (Mean ± SD)	LC_{50} (LCL–UCL) (ppm)	LC_{90} (LCL–UCL) (ppm)	χ^2
Methanol crude extract	125	4 ± 0[a]	1024.88	4357.76	1.84
	250	4.67 ± 1.15[a]	(700.10–2431.29)	(2004.92–9998.55)	
	500	34.67 ± 6.11[b]			
	1000	45.33 ± 8.32[c]			
	F-value	49.23***			
Hexane fraction	125	81.33 ± 4.61[a]	56.25	173.08	1.84
	250	96 ± 4[b]	(0–102.63)	(76.50–489.67)	
	500	100 ± 0[b]			
	1000	100 ± 0[b]			
	F-value	25.28*			
Chloroform fraction	125	5.67 ± 2.08[a]	504.10	1448.89	0.37
	250	22.67 ± 2.30[b]	(396.44–673.16)	(987.73–3032.94)	
	500	45.33 ± 6.11[c]			
	1000	81.33 ± 8.32[d]			
	F-value	110.36***			
Ethyl acetate fraction	125	0 ± 0[a]	8765.97	91807.37	0.50
	250	3.67 ± 0.57[b]	(–)	(–)	
	500	4.67 ± 1.15[b]			
	1000	10.67 ± 2.3[c]			
	F-value	33.57***			
Methanol fraction	125	3.67 ± 0.57[a]	3634.62	49677.64	0.11
	250	8.67 ± 1.15[a]	(#)	(#)	
	500	17.33 ± 6.11[b]			
	1000	25.33 ± 6.11[b]			
	F-value	14.38***			

Means within an extract followed by the same letter do not differ significantly at $p = 0.05$ (Duncan's test); *$p<0.05$; ***$p<0.001$; LC_{50} and LC_{90}: Lethal concentrations killing 50% and 90% of larvae, respectively; ppm: Parts per million; LCL: Lower confidence limit; UCL: Upper confidence limit; (–): No confidence limit estimated; (#): Very large values of confidence limit; Number of replicates: 4.

Table 3. Larvicidal activity of *C. rigidus* aqueous extract against IV instar larvae of *Aedes aegypti*, *Anopheles gambiae* and *Culex quinquefasciatus* 24 h post-treatment

Extract and fractions	Conc. (ppm)	% mortality (Mean ± SD)	LC_{50} (LCL–UCL) (ppm)	LC_{90} (LCL–UCL) (ppm)	χ^2
Aedes aegypti	250	0 ± 0[a]	761.26	1732.93	6.19
	500	26.67 ± 6.11[b]	(–)	(–)	
	1000	81.33 ± 4.61[c]			
	2000	85.33 ± 2.30[c]			
	F-value	328.97***			
Anopheles gambiae	250	0 ± 0[a]	1145.45	2655.19	1.14
	500	9.33 ± 2.3[b]	(929.14–1461.99)	(1947.69–4764.30)	
	1000	49.33 ± 4.61[c]			
	2000	76 ± 8[d]			
	F-value	166.02***			
Culex quinquefasciatus	250–2000	(#)	(#)	(#)	

Means within a mosquito species followed by the same letter do not differ significantly at $p = 0.05$ (Duncan's test); ***$p<0.001$; LC_{50} and LC_{90}: Lethal concentrations killing 50 and 90% of larvae, respectively; ppm: Parts per million; LCL: Lower confidence limit; UCL: Upper confidence limit; (–): No confidence limit estimated; (#): No mortality observed; Number of replicates: 4.

Table 4. Larvicidal activity of *C. rigidus* extract and fractions against IV instar larvae of *Anopheles gambiae* 24 h post-exposure

Extract and fractions	Conc. (ppm)	% mortality (Mean ± SD)	LC_{50} (LCL–UCL) (ppm)	LC_{90} (LCL–UCL) (ppm)	χ^2
Methanol crude extract	125	58.67 ± 4.61[a]	114.95	294.69	1.73
	250	78.67 ± 6.11[b]	(58.38–152.88)	(223.53–551.15)	
	500	100 ± 0[c]			
	1000	100 ± 0[c]			
	F-value	80.57***			
Hexane fraction	125	93.33 ± 2.3[a]	17.11	82.71	0.55
	250	100 ± 0[b]	(–)	(–)	
	500	100 ± 0[b]			
	1000	100 ± 0[b]			
	F-value	25*			
Chloroform fraction	125	16 ± 4[a]	445.56	1409.96	5.91
	250	16 ± 4[a]	(–)	(–)	
	500	45.33 ± 6.11[b]			
	1000	90.67 ± 6.11[c]			
	F-value	139.93***			
Ethyl acetate fraction	125	17.33 ± 4.61[a]	200.59	355.73	1.14
	250	62.67 ± 6.11[b]	(167.28–238.65)	(288.88–524.00)	
	500	100 ± 0[c]			
	1000	100 ± 0[c]			
	F-value	315.51***			
Methanol fraction	125	0 ± 0[a]	1854.12	5362.53	0.49
	250	0 ± 0[a]	(#)	(#)	
	500	8 ± 4[b]			
	1000	21.33 ± 6.61[c]			
	F-value	32.54***			

Means within an extract followed by the same letter do not differ significantly at $p = 0.05$ (Duncan's test); *$p<0.05$; ***$p<0.001$; LC_{50} and LC_{90}: Lethal concentrations killing 50 and 90% of larvae, respectively; ppm: Parts per million; LCL: Lower confidence limit; UCL: Upper confidence limit; (–): No confidence limit estimated; (#): Large values of confidence limit; Number of replicates: 4.

Table 5. Larvicidal activity of *C. rigidus* extract and fractions against IV instar larvae of *Culex quinquefasciatus* 24 h post-exposure

Extract and fractions	Conc. (ppm)	% mortality (Mean ± SD)	LC_{50} (LCL–UCL) (ppm)	LC_{90} (LCL–UCL) (ppm)	χ^2
Methanol crude extract	125	0 ± 0^a	447.38	766.86	
	250	12 ± 4^b	(379.72–531.38)	(626.32–1099.74)	1.93
	500	52 ± 4^c			
	1000	100 ± 0^d			
	F-value	765.5***			
Hexane fraction	125	0 ± 0^a	721.95	1121.70	0.02
	250	0 ± 0^a	(619.57–846.78)	(936.44–1591.71)	
	500	17.67 ± 2.08^b			
	1000	82.67 ± 4.61^c			
	F-value	721.43***			
CF, EAF, MF	125–1000	(#)	(#)	(#)	(#)

Means within an extract followed by the same letter do not differ significantly at $p = 0.05$ (Duncan's test); ***$p<0.001$; LC_{50} and LC_{90}: Lethal concentrations killing 50 and 90% of larvae, respectively; ppm: Parts per million; LCL: Lower confidence limit; UCL: Upper confidence limit; (#): No mortality observed; Number of replicates: 4.

killing relatively all exposed larvae at all concentrations. That made it once more the most efficient mosquito larvicide with LC_{50} value of as low as 17.11 ppm. MCE and EAF equally caused 100% mortality each at 500 ppm that resulted in getting LC_{50} values of 114.95 and 200.59 ppm for MCE and EAF, respectively. The AE obtained 76% mortality at 2000 ppm registering LC_{50} value of 1145.45 ppm (Table 3).

Only MCE and HF showed larvicidal activity against *Cx. quinquefasciatus*, with LC_{50} values of 447.38 and 721.95 ppm, respectively (Table 5). CF, EAF and MF did not cause mortality against IV instar larvae of *Cx. quinquefasciatus*. Likewise, AE was not effective against *Cx. quinquefasciatus* larvae (Table 3). No larvae for all the mosquito species survived in Diclorvos.

DISCUSSION

Previously, it has been well-recognized that plant extracts and phytochemicals could be developed into products suitable for mosquito control because many of them are selective, are often biodegradable, nontoxic products, and may be applied to mosquito breeding places in the same way as conventional insecticides[18]. Many plant extracts and essential oils possess larvicidal activity against various mosquito species[2, 8–11]. In the present study, different solvents extracts and fractions from Cameroonian *C. rigidus* leaf were studied for their toxicity against early IV instar larvae of yellow fever (*Ae. aegypti*), malaria (*An. gambiae*) and filariasis (*Cx. quinquefasciatus*) vectors and screened for phyto-constituents responsible of the toxicity. Hexane fraction was found to have the highest rate of mortality against all the target mosquito species in the present study, whereas in the case of other extracts and fractions, the concentrations had to be increased for better larvicidal effect. This corroborated with the earlier results obtained by Matasyoh *et al*[23] from *Aloe ngongensis* against *An. gambiae* larvae. Among the tested fractions, hexane fraction was the most active with LC_{50} value of 0.84 mg/ml followed by chloroform, acetone and ethyl acetate fractions with LC_{50} values of 0.98, 1.08 and 1.14 mg/ml, respectively. The methanol fraction was the less effective fraction to have LC_{50} value of 2 mg/ml. The larvae have respiratory siphon; they breathe through spiracles located on the 8th abdominal segment and therefore must come to the surface frequently to breathe. The HF used in this study had oil; hence, the oil could block the spiracles, resulting in asphyxiation and death of the larvae[24].

In the present study, aqueous extract was less effective against the three target mosquito species. The same result had been reported by Naveed and Muhammad[25] where the aqueous fraction showed lesser efficacy than hexane, chloroform, ethyl acetate, methanol and butanol fractions.

Saponins and alkaloids had been reported by Mousumi *et al*[26] to be responsible of toxicity of seed coat of *Cassia sophera* on all instar larvae of *Cx. quinquefasciatus*. According to a research, tannins and alkaloids in *Pistia stratiotes*; tannins, alkaloids and steroid glycosides in *Typha latifolia*; tannins, saponins and steroid glycosides in *Leucas martinicensi*; alkaloids, saponins and tannins in *Cynodon dactylon* and saponins and tannins in *Nymphaea lotus* have been reported to be responsible for larval toxicity of *Anopheles* mosquitoes[27]. In addition, triterpenoids and saponins in choroform; saponins in hexane; steroids, saponins, tannins and alka-

loids in methanol extracts of *Adansonia digitata* had revealed their toxicity against *Ae. aegypti* and *Cx. quinquefasciatus* larvae[28]. All the extract and fractions in the present study contained one or more phytochemical compounds. Therefore, the larvicidal activity might be due to the presence of those phytoconstituents.

In conclusion, the results of the present study revealed that the Cameroonian *C. rigidus* could be useful for managing field populations of *An. gambiae*, *Ae. aegypti* and *Cx. quinquefasciatus* vectors. Application of these extract and fractions to mosquito breeding habitats may lead to promising results in malaria and mosquito management programmes. However, further studies on the bio-guided fractionation of hexane fraction to bring out the most active molecule(s), their insecticidal mode of action, their effects on non-target organisms and the environment and formulations improving the insecticidal potency and stability are needed for their practical use as a naturally occurring mosquito larval control agent.

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