Original Research Article

Efficacy of the fractions of *Chenopodium ambrosioides* Linn (Chenopodiaceae) against *Anopheles gambiae*Giles and *Culex quinquefasciatus*Say larvae (Diptera: Culicidae)

Abstract

Botanical insecticides are nowadays highly encouraged in mosquito control programs because of their efficacy, specific target, biodegradability, less toxic for non-target species,and safe for the users. In this context, five fractions obtained from the spitting of *Chenopodium ambrosioides* leaf methanol extract were evaluated for their toxic effect against *Anopheles gambiae* and *Culex quinquefasciatus* larvae in the laboratory. The plant crude extract and its fractions were diluted in 1 mL of methanol and concentrations of 125ppm, 250ppm, 500ppm, and 1000 ppm were prepared in 100 mL solution with distilled water in the plastic cups. Bi-one was used at a unique concentration of 1000 ppm as positive control and 1 mL added to 99 mL of tap water was used as negative control. In each preparation test and control, 25 early fourth instar larvae of each mosquito species were transferred and larval mortality was recorded after 24 h. The phytochemical screening revealed the presence of phenolic compounds, alkaloids, terpenoids, flavonoids, and tannins in *C. ambrosioides* methanol extract and its 5 fractions. The plant methanol extract and its fractions caused a significant toxic effect against the 2 mosquito species larvae and fraction 1 was revealed as the most potent against both *An. gambiae* (CL_{50} = 66.39 ppm) and *Cx. quinquefasciatus* (CL_{50}= 251.41ppm) larvae. Thus, fraction 1 of *C. ambrosioides* might be used in small-scale potential mosquito breeding sites to reduce the density of the 2 mosquito species assessed around the buildings. This fraction 1 might be furthermore submitted to chromatography for the isolation of the compounds responsible for the larvicidal efficacy.

Keywords:*Chenopodium ambrosioides*, methanol extract, fractions, phytochemicals, *Anopheles gambiae, Culex quinquefasciatus*

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1. Introduction

Belonging to the family of Culicidae, mosquitoes are dipteran insects considered as the main public health problem in sub-Saharan Africa. These blood-sucking insects are involved in the transmission of numerous dreadful illnesses such as lymphatic filariasis, malaria, yellow fever, dengue fever, chikungunya, zika fever, etc[1].

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The mosquito species *Culex quinquefasciatus*,~~usual~~usually denselydistributed around human buildingsin the urban area and are a source of nuisance because of their unpleasant noises and painful bites[2, 3]. The mosquito speciesconstitutes also the most significant public health problemfor its involvement in the transmission ofJapanese encephalitis, West Nile fever, and lymphatic filariasis[4, 5]. In the sub-Saharan region of Africa, lymphatic filariasis affects more than 40 million people,andpersons with hydrocele or elephantiasis disabilities face sexual, marriage, educational, and employment discrimination[6, 7]

UNDER PEER REVIEW

*Anopheles gambiae*s.l. with its high blood-feeding behavior on human beings remains the main vector responsible of 98% of malaria transmission in sub-Saharan Africa [8]. According to the World Malaria Report of 2023, approximately 249 million malariacases were distributed in 85 malaria-endemic countries and 608,000 deaths were reported worldwide in 2022. About 93.6% of global cases and 95.4% of overall deaths are encountered in the WHO African Region in which children aged under 5 years represent 78.1% of total deaths recorded in 2022 [9]. In Cameroon, 6,459,013 malaria cases and 12,587 deaths were registered in 2022 [9].

To reduce the impact of these mosquito-borne diseases, several control methods were developed whether at the parasite level through chemotherapy procedure or vector control measures. Among these control methods,vector control by combining physical, biological, and chemical control remains a suitable measure since it has proven its effectiveness in numerous communities [10]. According to Antonio-Nkondjio et al. [11], mosquito vector control, especially anti-larval measures remainsremain an idealapproach since that method gets rid of these insects before reaching the adult stage\ and able, able to transmit diseases.However, cChemical mosquito control through the application of residual synthetic insecticides including organophosphates, carbamates, organochlorines, and pyrethroids is the most used[REFERNCES}. Unfortunately, the repeated misuse of these chemicals has led to the development of resistantdevelopment mosquitoes strains resistant to those insecticides[12, 13]. Besides, they pollute the environment and are toxic to humans, animals, and other non-target species[14]. As an alternative, plant-based insecticides might be suitable since they are biodegradable, target-specific, and less toxic for humans and animals [15, 16].

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The plant species *Chenopodium ambrosioides* (Chenopodiaceae) also called *Dysphaniaambrosioides*[REF] is an annual hermaphrodite plant, originated from America and largely distributed in tropical and sub-tropical regions,especially in Ghana, Senegal, Nigeria, and Cameroon[17]. As insecticide, larvicidal, and adulticidal efficacy of the methanol extract and essential oils of the plant werehave reportedbeen reported against different developmental stages of*An. gambiae*larvae and adults[18, 19]. The seeds and leaves of the plant were also toxic to the larvae and adults of *Anophelesarabiensis* and *An. gambiae*s.s.[20].Essential oil of that plant was reported to possess toxic effect against the grain-damage insects including *Sitophilus zeamais*, *Callosobruchus chinensis, C. maculatus, Acanthoscelides obtectus, Sitophilus granaries,* and*Prostephanustruncates* adults [21, 22]. Its powder was toxic for the Mexican bean weevil *Zabrotessubfasciatus*[23].

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From our knowledge, no previous study was reported on the larvicidal activity of *C. ambrosioides* fractions against *An. gambiae* and *Cx. quinquefasciatus* larvae. This present study aimed to evaluate the effectiveness of *C. ambrosioides* leaf fractions againstyouare the larvae of *An. gambiae* and *Cx. quinquefasciatus*,respectively the main vectors of malaria and lymphatic filariasis.

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2. Materials and Methods

2.1. Harvesting ~~of the~~and identification of plant material

The green leaves of *C. ambrosioides* were collected early in the morning (6:00 +1h GMT) from Bini-Dang (latitude 7°24.949'N, longitude 13°32.870'E and altitude 1093m) in Adamaoua region of Cameroun. The plant species was identified by Prof Mapongmetsem Pierre-Marie, the botanist of the Faculty of Science, University of Ngaoundere, Cameroon. The voucher of the plant was deposited at the National Herbarium of Cameroon of Yaounde and its identity was confirmed under the registration number of No. 44452/NHCam in comparison with the sample of Asonganyi J.N.no.233.

2.2. Processing of the plant leaves

The plant leaves were washed with tap water and dried under shade in the room at ambient temperature (25±3℃; 75±4% r.h) for 14 days. Then, the dried leaves were ~~reduced in the~~grind into powder in a~~the~~ wooding mortar and sieved with 0.4 mm mesh size. The obtained plant powder was stored in dark sealed plastic bags in the refrigerator set at -4℃ until their use for methanol extraction.

2.3. Plant extraction with methanol solvent

For methanolic extract preparation, 750 g of *C. ambrosioides* powder was soaked in 3000mL of methanol solvent for 72 h. During the process, the maceration was manually stirred twice a day (morning and afternoon). After 72 h of maceration, the supernatant was filtered using Whatman No.1 filter paper. The remained residue was rinsed and filtered several times with fresh methanol until a clear phase was observed. The filtrate obtainedwas concentrated using a rotary evaporator and completely dried in the oven set at 60℃. The methanolic extract obtained was weighed and kept at -4℃ in the refrigerator. The extraction yield was calculated according to the following formula.

$$\text{Extraction yield (\%)} = \frac{\text{Weight of plant methanolic extract obtained}}{\text{Weight of the plant powder used}} \times 100$$

2.4. Column chromatography fractionation

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To fractionate the methanolic extract of *C. ambrosioides*, a glass column chromatography (8 cm diameter and 45 cm height) was used. Previously, 60 g of the crude extract dissolved in 50 mL of methanol was fixed on 200 g of silica gel (63-200 µm particle size) and then transferred into the column containing already 400 g of silica gel. The column elution started with the non-polar solvent hexane followed by the gradual increase of the solvent system polarity. However, the column chromatography was eluted with the solvent system of 100% hexane followed by 95% hexane + 5% ethyl acetate, then 90% hexane + 10% ethyl acetate, 85% hexane + 15% ethyl acetate, 50% hexane + 50% ethyl acetate and 75% hexane + 25% ethyl acetate systems. The following solvent system was 100% ethyl acetate, then 50% ethyl acetate + 50% methanol system, and at last 100% methanol system. During the column elution process, 500 mL of the filtrate fraction was recovered. At the end of the column elution process, 55 fractions were collected and immediately concentrated using a rotary evaporator. To pool the 55 fraction samples obtained, each dry fraction obtained was submitted to the thin layer chromatography (analytical TLC) resulting to 5 major fractions. The yield of each major fraction was calculated using the following formula:

$$\text{Fraction yield (\%)} = \frac{\text{Weight of the major fraction obtained}}{\text{Weight of the methanolic crude extract used}} \times 100$$

2.5. Phytochemical screening of extract and fractions of*C. ambrosioides*

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Extract and fractions of *C. ambrosioides* were screened to detect the presence of alkaloids, flavonoids, saponins, tannins, polyphenols, sterols, and terpenoids in these products following the method described by Harborne [24]. These 7 phyto-constituents were targeted for screening based on their insecticidal properties largely reported in the previous studies.

2.6. Collection and rearing of mosquito species

Egg rafts of *Cx. quinquefasciatus* were collected from the strain established in the insectarium of the Laboratory of Applied Zoology, Faculty of Science, Faculty of Science, University of Ngaoundere, Cameroon while *An. gambiae* eggswere provided by the Organization of Coordination for the Fight against Endemic Diseases in Central Africa (OCEAC) in Yaounde, Cameroon. In the insectarium, the eggs of each mosquito species were transferred into plastic trays containing tap water to hatch into larvae. Each mosquito species larvae were reared according to the standard WHO [25]protocol in the laboratory (25±2°C; 78±4% r.h.). Larvae of *An. gambiae* were fed with TetraMin Baby food while the mixture of crayfish and biscuit (1:3 ratio) was made to feed *Cx. quinquefasciatus* larvae. To avoid the suffocation of the mosquito larvae caused by the decayed food, water in each tray was

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renewed every 2 days. Fourth instar larvae of each mosquito species were used for the larvicidal bioassay.

2.7. Larvicidal test

The larvicidal test of the extract and fractions of *C. ambrosioides* on *An. gambiae* and *Cx. quinquefasciatus*larvae was conducted in the insectarium of the Laboratory of Applied Zoology, Faculty of Science, University of Ngaoundere, Cameroon according to the protocol of WHO [26]. Previously, stock solutions (in total volume of 20 mL each) of theplant methanolic extract or its fractionswere prepared with methanol solvent. Then from each stock solution, concentrations of 1000, 500, 250, and 125 ppm in four replicates each were prepared in a volume of 100 mL with tap water in plastic cups (250 mL). The commercial insecticide Bi-One® (1000 ppm) was tested as the positive control while the negative control consisted of 1 mL methanol added to 99 mL tap water. Then, a batch of twenty-five (25) 4th instar larvae of *An. gambiae* or *Cx. quinquefasciatus*were transferred separately into each prepared concentration solution and controls and larval mortality was recorded after 24 h post-exposure. The larval mortality was corrected withthe Abbott [27]formula when mortality in the negative control ranged from 5% and 20% as follows:

$$\text{Mortality rate (\%)} = \frac{\text{Number of dead larvae in tests or controls}}{\text{Total number of mosquito larvae used}} \times 100$$

$$\text{Corrected mortality (\%)} = \frac{NT - NC}{1 - NC} \times 100$$

Where NT= Number of death larvae in the test, NC= Number of death larvae in negative control

2.8. Statistical analysis

Data of the corrected larval mortality were transformed into the percentage of mortality using Microsoft Excel 2019 and submitted to analysis of variance using SPSS (Statistical Package for Social Sciences) version 16.0 software. For means comparison, Tukey's test (P = 0.05) was applied. To determine the concentration values of the plant extract or fractions causing 50% (LC_{50}) and 90% (LC_{90}) larval mortality, Probit analysis [28]was employed.

3. Results

3.1.Extraction yields

Cold maceration during 72h of 750 g of *C. ambrosioides* in 3000 mL of methanol solvent yielded 12.60% of extract which after splitting, yielded different fraction proportions as presented in Ttable 1. After the partition of 60 g of *C. ambrosioides*methanolic extract using column chromatography, 5 major fractions were obtained in which fraction 4 (48.06%) and

UNDER PEER REVIEW

fraction 1 (45.32%) presented high yields compared to the other fractions. However, fraction 5 yielded a moderate yield of 5.60% while very low yields were registered in fraction 2 (0.44%) and fraction 3 (0.40%).

Table 1. Extraction yields of the leaf extract and fractions of *Chenopodiumambrosioides*

Extract/Fraction	Weights (g) of extract/fraction obtained	Yield (%)
Methanol extract	189.00	12.60[a]
Fraction 1	27.19	45.32[b]
Fraction 2	0.26	0.44[b]
Fraction 3	0.24	0.40[b]
Fraction 4	29.16	48.06[b]
Fraction 5	3.36	5.60[b]

[a]obtained from 750g of plant powder used and [b]obtained from 60g of methanol extract used.

3.2. Phytochemical constituents of *Chenopodiumambrosioides* extract and fractions

In the crude methanolic extract of *C. ambrosioides*, tannins, terpenoids, flavonoids, polyphenols, and alkaloids were present in variable concentrations (Table 2). After the fractionation of the plant crude extract, alkaloids were present in fractions 1, 2, and 3 while polyphenols were found in fraction 1 and fraction 3. Flavonoids were present in all 5 fractions obtained while terpenoids and tannins were only found in fraction 1 and fraction 4 (Table 2).

Table 2. Phytochemical constituents of *Chenopodiumambrosioides* extract and its 5 fractions

Phytochemicals	Crudeextract	Fraction1	Fraction2	Fraction3	Fraction4	Fraction5
Alkaloids	+	+	+	+	-	-
Polyphenols	+	+	-	+	-	-
Flavonoids	+	+	+	+	+	+
Terpenoids	+	+	-	-	+	-
Steroids	-	-	-	-	-	-
Tannins	+	+	-	-	+	-
Saponins	-	-	-	-	-	-

+ = present and - = absent

3.3. Effect of *Chenopodiumambrosioides* extract/fractions on*Anopheles gambiae* larvae

Figure 1 presents the mortality rate of *An. gambiae* larvae exposed to the methanolic crude extract and the 5 fractions of *C. ambrosioides*. In general, the plant methanolic extract and its 5 fractions caused a significant concentration-dependent larvicidal activity against that malarial vector. *C. ambrosioides*methanolic extract exhibited a significant (F=241.53, df1=5, df2=12, P<0.001) larvicidal activity varying from 32% at the lowest dose of 125 ppm to 100% at the highest dose of 1000 ppm. After splitting the plant crudeextract fraction 1 induced a high mosquito larval mortality ranging significantly (F=450.75, df1=5, df2=12; P<0.001) from 75% (at 125 ppm) to 100% (at 1000 ppm). Fraction 2 of the plant caused a

UNDER PEER REVIEW

moderate mortality *An. gambiae* larvae ranging significantly (F=669.03, df1=5, df2=12; P<0.001) from 0.0% (at 125 ppm) to 85.33% (at 1000 ppm). Fraction 3 caused also a high larvicidal activity varying significantly (F=431.14, df1=5, df2=12; P<0.001) from 37.33% (at 125 ppm) to 100% (at 1000 ppm). A moderate larvicidal activity ranged significantly (F=253.85, df1=5, df2=12; P<0.001) from 5.33% (at 125 ppm) to 53.33% (at 1000 ppm) was recorded with fraction 4.Fraction 5 exhibited also moderate larval mortality varying significantly (F=319.80, df1=5, df2=12; P<0.001) from 0.0% (at 125 ppm) to 70.66% (at 1000 ppm).

The fractionation of the methanolic crude of *C. ambroides* significantly improved the efficacy of the plant against the larvae of *An. gambiae* since some fractions were revealed as more effective than the crude extract (Table 3). Among the fractions of *C. ambroides* obtained and tested on *An. gambiae* larvae, fraction 1 (LC_{50}= 66.39 ppm) was revealed as the most potent followed by fraction 3 (LC_{50}= 146.22 ppm), the methanolic crude extract (LC_{50}= 190.35 ppm), fraction 2 (LC_{50}= 525.33 ppm), fraction 5 (LC_{50}= 620.18 ppm) and fraction 4 (LC_{50}= 914.18 ppm) (Table 3).

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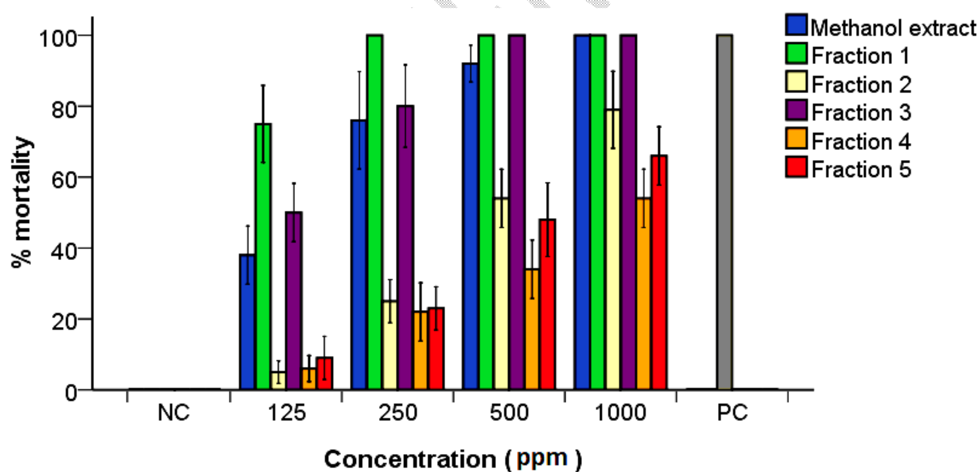

Figure 1. Mortality rate of *Anopheles gambiae* fourth instar larvae exposed for 24h to the methanol extract and fractions of *Chenopodium ambrosioides* in the laboratory. NC=negative control (1mL of methanol + 99 mL of tap water), PC= positive control (Bi-Onetested at 1000 ppm).

Table 3. LC_{50} and LC_{90} values (ppm) after 24h of *Chenopodium ambrosioides* extract and fractions against *Anopheles gambiae* fourth instarlarvae in laboratory conditions.

Plant products	Slope±SE	R^2	LC_{50}(95% CI)	LC_{90}(95% CI)	χ^2

ME	2.93±0.16	0.89	190.35 (164.51-215.62)	520.02 (440.54-651.35)	24.08**
F1	2.93±0.37	0.66	66.39 (30.99-91.09)	181.40 (150.03-233.14)	25.23**
F2	4.93±0.25	0.93	525.33 (448.08-613.31)	955.17 (788.92-1307.12)	74.12***
F3	5.01±0.34	0.70	146.22 (137.98-154.13)	263.41 (245.25-287.53)	8.55^{ns}
F4	1.91±0.13	0.97	914.18 (804.59-1068.44)	4269 (3186.69-6266.43)	13.73^{ns}
F5	3.91±0.21	0.91	620.18 (512.53-769.80)	1317 (1000.33-2216.87)	85.99***

***P<0.001 ; *P<0.05 ;^{ns}P>0.05 ;F= Fraction ; CI= Confident Interval;MCE= MethanolicCrudeExtract; LC= Lethal Concentration; R^2= Coefficient of Determination ; χ^2= Chi-square ; SE = Standard Error.

3.4. Effect of *Chenopodiumambrosioides* extract andfractions against *Culexquinquefasciatus* larvae

The mortality rate after 24h of *Cx. quinquefasciatus* larvae treated with *C. ambrosioides* methanolic extract and its 5 fractions are presented in Figure 2. Globally, the plant crude extract and its fractions exhibited a significant concentration-dependent mortality of that mosquito species, and that mortality augmented with the increasing concentrations. The plant methanolic extract caused a significant (F=485.54, df1=5, df2=12, P<0.001) larvicidal activity ranging from 0.0% at the lowest concentration of 125 ppm to 100% at the highest dose of 1000 ppm.After fractionation of *C. ambrosioides*extract, its fraction 1 exhibited high larval mortality varying significantly (F=389.37, df1=5, df2=12; P<0.001) from 10.0% (at 125 ppm) to 100% (at 1000 ppm).Fraction 3 of the plant caused a moderate mortality *Cx. quinquefasciatus* larvae ranging significantly (F=751.40, df1=5, df2=12; P<0.001) from 0.0% (at 125 ppm) to 57.33% (at 1000 ppm).Others fractions induced low mortality of mosquito larvae varying significantly (F=418.28, df1=5, df2=12; P<0.001) from 0.0% (at 125 ppm) to 33.33% (at 1000 ppm) for fraction 2;significantly (F=748.34, df1=5, df2=12; P<0.001) from 0.0% (at 125 ppm) to 34.66% (at 1000 ppm) for fraction 4; and significantly (F=1016.0, df1=5, df2=12; P<0.001) from 0.0% (at 125 ppm) to 16.00% (at 1000 ppm) for fraction 5.

Values of LC_{50} and LC_{90} of *C. ambrosioides*extract and fractions presented in Table 4 highlighted fraction 1 (LC_{50}=251.41 ppm and LC_{90}=534.97 ppm) as the most potent on *Cx. quinquefasciatus*larvae compared to other fractions tested. Fraction 3 (LC_{50}=930.37 ppm) and crude extract (LC_{50}=989.68 ppm) caused moderate activity while fraction 4 (LC_{50}=1272.31 ppm), fraction 2 (LC_{50}=1520.45 ppm) and fraction 5 (LC_{50}=4482.97 ppm) exhibited a low activity against *Cx. quinquefasciatus*larvae (Table 4).

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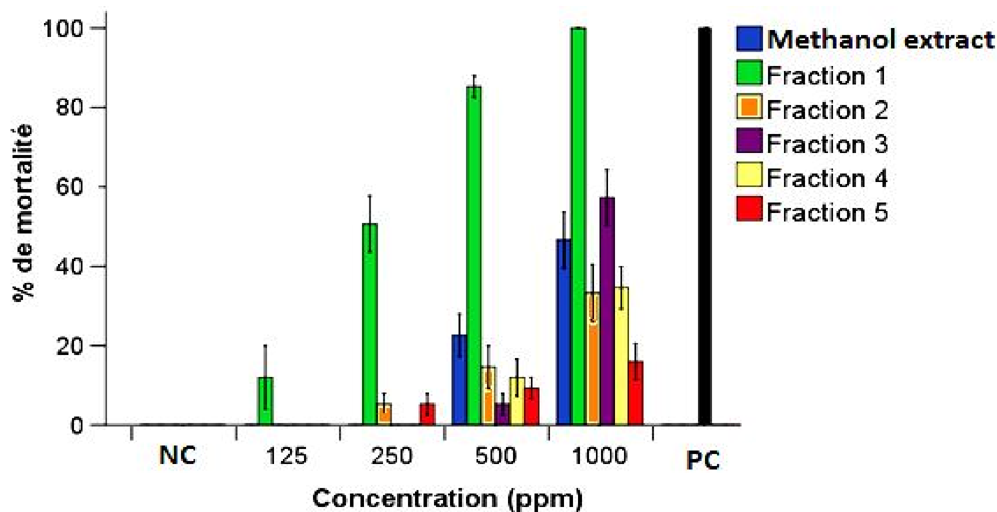

Figure 2. Mortality percentage after 24h of *Culex quinquefasciatus*fourth instar larvae exposed to the extract methanol and fractions of *Chenopodium ambrosioides*in the laboratory. NC=negative control (1mL of methanol + 99 mL of tap water), PC= positive control (Bi-One 1000 ppm).

Table 4. LC_{50} et LC_{90} (ppm) values after 24h of *Chenopodium ambrosioides* extract and fractions tested against *Culex quinquefasciatus*fourth instar larvae in the laboratory conditions.

Plant products	Slope±SE	R^2	LC_{50}(95% CI)	LC_{90}(95% CI)	χ^2
MCE	3.35±0.24	0.97	989.68(857.42-1211.99)	2383.38(1784.67-3840.56)	25.32**
F1	3.90±0.19	0.88	251.41(230.58-273.52)	534.97(474.93-621.29)	18.03ns
F2	2.22±0.19	0.96	1520.45(1281.18-1914.58)	5736.53(4038.86-9386.84)	13.36ns
F3	5.88±0.44	0.94	930.37(886.13-982.29)	1536.08(1401.03-1731.77)	5.22ns
F4	3.29±0.29	0.97	1272.31(1136.41-1478.50)	3114.27(2467.48-4325.50)	12.45ns
F5	1.44±0.19	0.91	4482.97(2780.14-10096.65)	34749.17(14119.33-165004.22)	14.02ns

**P<0.01; ^{ns}P>0.05;F= Fraction ; CI= Confident Interval; MCE= MethanolicCrudeExtract; LC= Lethal Concentration; R^2= Coefficient of Determination ; χ^2= Chi-square ; SE = Standard Error.

4. Discussion

From numerous previous studies, plant extracts, fractions, and isolated compounds as well as essential oils have been reported to possess insecticidal properties[29, 30]. Research on these botanical insecticides is regarded nowadays as an alternative to the synthetic insecticides currently in use. This,is because botanical-based insecticides are effective, target-specific, reduce insect resistance issues, environmentally less toxic, and are safe for the users[31].

In this present study, the methanol extract of *C. ambrosioides* and its 5 fractions caused a significant concentration-dependent larvicidal activity against larvae of *An. gambiae* and *Cx. quinquefasciatus*.Similarly, Oumarou et al. [18]reported a potential efficacy of the

methanolic extract of *C. ambrosioides* extract 24 h post-treatment against larvae of *An. gambiae* with LC_{50}= 204.56 ppm. *Chenopodium suaveolens* ethanol extract was reported to exhibit a high larvicidal effect against *Anopheles* species larvae with LC_{50} of 389.05 mg/L after 24 h[32].The leaf ethanolic extract *C. ambrosioides* was highly toxicagainst the fourth instar larvae of the invasive Zika and dengue virus*Aedes albopictus* with LC_{50}value of 199.55 ppm [33]. N-hexane extract of *C. botrys*whole-plant exhibited remarkable larvicidal activity against*Cx.quinquefasciatus* larvae with a 24-hour lethal concentration (LC_{50}) of 495.6 ppm [34]. From Nigeria, ethanolic extract of *C. ambrosioides* was found toxic for*Sitophilus zeamais* (LC_{50} =0.04 g/L), *Triboliumcastaneum* (LC_{50} =0.04 g/L) and *Callosobruchus maculatus* (LC_{50} =0.02 g/L) after 48h[35]. *C. ambrosioides*ethanolic extract was also toxic to sweetpotato whitefly,*Bemisiatabaci* which tested at 6% concentration caused 93% mortality of the insect pest [36]. The efficacy of the plant extract against mosquito larvae could be due to their relative richness in phytochemicals like tannins, flavonoids, alkaloids, terpenoids, and phenolic groups. Besides, plant parts, solvents, and extraction methods might also influence the effectiveness of the plant extract [37].

Among the fractions of *C. ambrosioides* tested against *An. gambiae* larvae in this present study, fraction 1 was revealed as the most potent on mosquito larvae. In the same way, fractionated extracts of *Dracaena loureiri* endocarp evaluated on *Cx.quinquefasciatus* and *Anopheles minimus*revealed fractionated group extracts RC-DT 012 (LC_{50} = 0.66 mg/L) and RC-DT 013 (LC_{50} = 0.94 mg/L) as the most potent against *Cx.quinquefasciatus*mosquito species [38]. Column chromatographic fractions F1 and F3 of the methanol extract of *Calpurnia aurea* showed remarkable larvicidal activity against *An. arabiensis* with LC_{50} of 62.51 and 82.33 ppm, respectively [39]. Fractions F2 and F3 of *Ricinus communis*demonstrated a high larvicidal activity against *An. arabiensis* larvae with the lowest LC_{50} of 21.012 and 28.410 ppm [40].

The high larvicidal efficacy of fraction 1 should be linked to the non-polar hexane solvent mostly used for its elution. Numerous previous studiesreported hexane fraction as the most effective against mosquito species. It is the case of Bouba et al.[41]who reported the hexane fraction of *Cyperus rotundus*(LC_{50}=52.43 ppm) ppm slightly more effective than ethylacetate fraction (LC_{50}=54.26 ppm) that plant against *An. arabiensis* larvae.Similarly, among the fractions tested, the n-hexane fraction was most effective against *Cx. quinquefasciatus* (LC_{50}= 3394.9 ppm) and *An. gambiae* (LC_{50}= 385.9 ppm) larvae [42]. N-hexane (LC_{50}=298.8 ppm) fraction of *Annona senegalensis* was also found to be more toxic than other fractions

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UNDER PEER REVIEW

against*An. gambiae* (LC_{50}=2087.6 ppm), and *Cx. quinquefasciatus*(LC_{50}=2087.6 ppm) larvae [43].

The effectiveness of fraction 1 might linked to the high amount and diversity of secondary metabolites found in this fraction compared to the plant fractions and crude methanolic extract. Secondary metabolites such as alkaloids, polyphenols, terpenoids, flavonoids, and tannins might actlike carbamates and organophosphates which have demonstrated some interference of acetylcholinesterase inhibition in the central nervous system of the insect through oral, respiratory, or cutaneous absorption, and consequently leading to seizures and death of insect pest by intoxication [44]. According toTanaka[45], these secondary metabolites may act on the γ-Aminobutyric acid (GABA)system, leading to the inhibition of mitochondrial activity. Recent studies have revealed that *Aacetogeninsquamocin* affects the digestive cells of the midgut by damaging the anal papillae of *Ae. aegypi*larvae causing cell death by autophagy [46, 47]. It has also been observed that flavonoidsand alkaloidscan strongly inhibit the Acetylcholine esterase of mosquito larvae and act as a growth regulator [48, 49]. They influence the central nervous system of the insects, by acting on the receptors of several neurotransmitters, provoking uncontrolled muscular movements, paralysis, seizures, and death [50]. Besides, morphological examination of dead *Aedes albopictus*and *Ae. aegypti* larvae previously exposed to confertifolin isolated from *Polygonum hydropiper*showed the destruction ofthe anal zone of the larvae with the loss of ridge-like reticulum and also the damage ofthe anal papillae, with a shrunk of the cuticle bordered [51, 52].Moreover, serial damages of the epithelium layer and peritrophic matrix tissues of the midgut portion of *Ae. aegypti* and *Cx. quinquefasciatus* larvae after exposition to *Leonotisnepetifolia*-mediated silver nanoparticles were reported by [53].

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5. Conclusion

The methanol extract of *C. ambrosioides* was significantly toxic against the larvae of *An. gambiae* and *Cx. quinquefasciatus.* But, the partition of the methanol extract of the plant conducted to five major fractions and fraction 1 was highly rich in some phytochemicals including flavonoids, alkaloids, phenolics, terpenoids, and saponins. However, fraction 1 (CL_{50}= 66.39 ppm) was revealed as the most toxic on *An. gambiae* and while fraction 3 (CL_{50}= 930.37 ppm) was the most potent against *Cx. quinquefasciatus*larvae. Thus, fraction 1 and fraction 3 may be used as phyto-larvicides to control *An. gambiae*larvae in their breeding sites around the human habitations. These two fractions might be furthermore

UNDER PEER REVIEW

submitted to column chromatography for the isolation of the compounds responsible of the larvicidal efficacy.

Data Availability

Data used to support the findings of this present study are included in the manuscript.

References

[1] Manikandan S, Mathivanan A, Bhagyashree B, Hemaladkshmi P, Abhisubesh V, Poopathi S. A review on vector-borne disease transmission: Current strategies of mosquito vector control. Indian J Entomol. 2023; 85(2): 503-513. https://doi.org/10.55446/IJE.2022.593.

[2] Jones C, Machin C, Majambere K, Ali S, Khatib A, Mcha O, Ranson H, Kelly-Hope LA. Insecticide resistance in *Culex quinquefasciatus* from Zanzibar: implications for vector control programmes. Parasites &Vect. 2012; 5(78): 1-9.https://doi.org/10.1186/1756-3305-5-78.

[3] Antonio-Nkondjio C, Defo-Talom B, Tagne-Fotso R, Tene-Fossog B, Ndo C, Lehman LG, Tchuinkam T, Kengne P, Awono-Ambene P. High mosquito burden and malaria transmission in a district of the city of Douala, Cameroon. *BMC* Infect Dis 2012; 12(175): 1-8. https://doi.org/10.1186/1471-2334-12-275

[4] Van den Hurk AF, Hall-Mendelin S, Jansen CC, Higgs S. Zika virus and *Culex quinquefasciatus* mosquitoes: a tenuous link. The Lancet Infect Dis. 2017; 17(10):1014-1016. https://doi.org/1016/S1473-3099(17)30518-2.

[5] Rai P, Bharati M, Subba A, Saha D. Insecticide resistance mapping in the vector of lymphatic filariasis, *Culex quinquefasciatus* Say from northern region of West Bengal, India. PLoS ONE 14(5): e0217706. https://doi.org/10.1371/journal.pone.0217706.

[6] Nchoutpouen E, Talipouo A, Djiappi-Tchamen B, Djamouko-Djonkam L, Kopya E, Ngadjeu S, Doumbe-Belisse P, Awono-Ambene P, Kekeunou S, Wondji CS, Antonio-Nkondjio C. *Culex* species diversity, susceptibility to insecticides and role as potential vector of Lymphatic filariasis in the city of Yaoundé, Cameroon. PLoS Neglect Trop Dis. 2019; 13(4):e0007229. doi: 10.1371/journal.pntd.0007229.

[7] Babu BV, Mishra S, Nayak AN. Marriage, sex, and hydrocele: an ethnographic study on the effect of filarial hydrocele on conjugal life and marriageability from Orissa, India. PLoSNeglect Trop Dis. 2009; 3(4): e414. https://doi.org/10.1371/journal.pntd.00004143:e414,

[8] WHO. World malaria report 2021. Geneva: World Health Organization; 2022. Licence: CC BY-NC-SA 3.0 IGO, 2022.

[9] WHO, World malaria report 2022. Geneva: World Health Organization; 2023. Licence: CC BY-NC-SA 3.0 IGO, 2023.

[10] WilsonAL, CourtenayO, Kelly-HopeLA, ScottTW, TakkenW, TorrSJ, LindsaySW. The importance of vector control for the control and elimination of vector-borne diseases.PLoSNeglect Trop Dis. 2020; 14(1): e0007831. https://doi.org/10.1371/journal.pntd.0007831

[11] Antonio-NkondjioC, SandjoNN, Awono-AmbeneP, WondjiCS. Implementing a larviciding efficacy or effectiveness control intervention against malaria vectors: key parameters for success.Parasites &Vect. 2018; 11(57): 1-12. https://doi.org/10.1186/s13071-018-2627-9.

[12] Yewhalaw D, Kweka EJ. Insecticide resistance in East Africa—history, distribution and drawbacks on malaria vectors and disease control. In: Trdan S, editor. Agricultural and

Biological Sciences. Insecticides resistance. Rijeka: Intech; 2016: 189-215.https://doi.org/10.5772/61570.
[13] YaredS, GebressielasieA, DamodaranL, BonnellV, LopezK, JaniesD, CarterTE. Insecticide resistance in *Anopheles stephensi* in Somali Region, eastern Ethiopia.Malarial J. 2020; 19(180): 1-7. https://doi.org/10.1186/s12936-020-03252-2.
[14] TudiM, RuanDH, WangL, LyuJ, SadlerR, ConnellD, ChuC, PhungDT. Agriculture Development, Pesticide Application and Its Impact on the Environment.Int J Environ Res Pub Health. 2021;18(3):1112. https://doi.org/10.3390/ijerph18031112.
[15] ShaalanEAS, CanyonD, YounesMWF, Abdel-WahabH, MansourAH.A review of botanical phytochemicals with mosquitocidal potential.Environ Int. 2005; 31(8):1149-1166. https://doi.org/10.1016/j.envint.2005.03.003.
[16] PavelaR.History, presence and perspective of using plant extracts as commercial botanical insecticides and farm products for protection against insects - a review.Plant Protect Sci. 2016; 52(4):229-241. https://doi.org/10.17221/31/2016-PPS.
[17] KueteV. Chapter 22-Physical, hematological, and histopathological signs of toxicity induced by African medicinal plants. In: Toxicological survey of African medicinal plants. Elsevier. 2014: 635-657. https://doi.org/10.1016/b978-0-12-800018-2.00022-4.
[18] Oumarou KM, Younoussa L, Nukenine EN.Toxic effect of *Chenopodium ambrosioides, Hyptissuaveolens* and *Lippiaadoensis* leaf methanol extracts and essential oils against fourth instar larvae of *Anopheles gambiae* (Diptera: Culicidae).Int J MosqRes. 2018; 5: 61-66. https://www.dipterajournal.com/archives/2018/5/1/A/5-1-7.
[19] Oumarou KM, Younoussa L, Langsi JD, Saotoing P, Nukenine EN. Adulticidal activity of *Hyptissuaveolens*, *Chenopodium ambrosioides* and *Lippiaadoensis* Leaf extracts and essential oils against *Anopheles gambiae* (Diptera: Culicidae).Current J ApplSciTechnol. 2021; 40(2): 18-32. https://doi.org/10.9734/CJAST/2021/v40i231249.
[20] Bigoga JD, Petola A, Saahkem SA, Ndindeng JLN, Ngondi JL, Nyegue M, Oben JE, Leke RGF. Larvicidal and repellent potential of *Chenopodium ambrosioides* Linn essential oil against *Anopheles gambiae* Giles (Diptera: Culicidae).The Open Entomol J. 2013; 7: 16-22. https://doi.org/10.2174/1874407901307010016.
[21] Tapondjou LA, Adler C, Bouda H, Fontem DA. Efficacy of powder and essential oil from *Chenopodium ambrosioides* leaves as post-harvest grain protectants against six-stored product beetle.J Stored Prod Res. 2002; 38(4): 395-402.https://doi.org/10.1016/S0022-474X(01)00044-3.
[22] Langsi DJ. Nukenine EN, Fokunang CN, Suh C, Goudoungou WJ. Potentials of essential oils of *Chenopodium ambrosioides* L. and *Cupressus sempervirens* L. against stored maize pest, *Sitophilus zeamais*Motschulsky.J EntomolZool Stud.2017; 5(2): 309-313. https://www.entomoljournal.com/archives/?year=2017&vol=5&issue=2&part=E&ArticleId=1607.
[23] SelaseAG, GetuE. Evaluation of botanical plants powders against *Zabrotes* haricot beans under laboratory condition.AfrJ Agric Res. 2009; 4: 1073-1079. Available online at http://www.academicjournals.org/AJAR
[24] HarborneJB. Texbook of phytochemical methods. Guide to modern techniques of plant analysis", 5th Edition, Chapman and Hall Ltd, London, 2008: 21-72.
[25] WHO. Guidelines for laboratory and field testing of mosquito larvicides. World Health Organization, Geneva, Switzerland, 2005.
[26] WHO. Pesticides and their application for the control of vectors and pests of public health importance: World Health Organization, WHO Pesticides Evaluation Scheme. 2006: 113.
[27] AbbottWS. A method of computing the effectiveness of an insecticide.J EconEntomol. 1925; 18(2): 265-267.https://doi.org/10.1093/jee/18.2.265a.

UNDER PEER REVIEW

[28] FinneyDJ. Probit analysis. 3rd edition, Cambridge University Press, London. 1971: 68-72.

[29] PavelaR, MaggiF, IannarelliR, BenelliG.Plant extracts for developing mosquito larvicides: From laboratory to the field, with insights on the modes of action. Acta Tropica. 2019; 193: 236-271. https://doi.org/10.1016/j.actatropica.2019.01.019.

[30] WangrawaDW, YaméogoF, SombiéA, EsalimbaE, OchomoE, BorovskyD, BadoloA, SanonA.Methanol and acetone extracts from the leaves of selected aromatic plants affect survival of field collected Anopheles arabiensis (Diptera: Culicidae) from Kisumu, Kenya. J Med Entomol. 2023; 60(5): 1030-1037. https://doi.org/10.1093/jme/tjad066.

[31] PavelaR.Essential oils for the development of eco-friendly mosquito larvicides: A review. Indust Crops Prod. 2015; 76(2): 174-187. https://doi.org/10.1016/j.indcrop.2015.06.0.

[32] DawetA, IkaniA, YakubuDP.Larvicidal effects of *Hyptissuaveolens* and *Chenopodium ambrosioides* on *Anopheles* mosquito larvae.Int J Sci Eng Res. 2016; 7(1): 456-470.

[33] Subramaniam J, MuruganK, JebanesanA, PontheckanP, DineshD, NicolettiM, WeiH, HiguchiA, KumarS, CanaleA, GiovanniGB. Do *Chenopodium ambrosioides*-Synthesized silver nanoparticles impact *Oryziasmelastigma* predation against *Aedes albopictus* Larvae? J Clust Sci. 2017; 28: 413-436. https://doi.org/10.1007/s10876-016-1113-9.

[34] IlahiaI, YousafzaiAM, AttaullahM, HaqTU, RahimA, KhanW, KhanAA, UllahS, JanT, KhaneMM, RahimG, ZamanN. Mosquitocidal activities of *Chenopodium botrys* whole plant n-hexane extract against *Culex quinquefasciatus*.Braz J Biol. 2023; 83: e240842. https://doi.org/10.1590/1519-6984.240842.

[35] DenloyeAA, MakanjuolaWA, TeslimOK, AlafiaOA, KasaliAA, EshilokunAO. Toxicity of *Chenopodium ambrosioides* l. (Chenopodiaceae) products from Nigeria against three storage insects.J Plant Protect Res. 2010; 50(3): 379-384.https://doi.org/10.2478/v10045-010-0064-7.

[36] Vite-VallejoO, Barajas-FernándezMG, Saavedra-AguilarM, Cardoso-TaketaA. Insecticidal effects of ethanolic extracts of *Chenopodium ambrosioides*, *Piper nigrum*, *Thymus vulgaris*, and *Origanum vulgare* against *Bemisiatabaci*,Southwestern.Entomol. 2018; 43(2): 383-393. https://doi.org/10.3958/059.043.0209.

[37] SultanaB, AnwarF, AshrafM. Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts.Molecules. 2009; 14(6): 2167-2180. https://doi.org/10.3390/molecules14062167.

[38] ThongwatD, ChokchaisiriR, GanranooL, BunchuN. Larvicidal efficacy of crude and fractionated extracts of *Dracaena loureiri*Gagnep against *Aedes aegypti, Aedes albopictus, Culex quinquefasciatus*, and *Anopheles minimus* mosquito vectors.Asian Pac J Trop Biomed. 2018; 8(5): 273-278. https://doi.org/10.4103/2221-1691.233009

[39] EukubayA, DegagaE, AregaE, TeferaMH. Larvicidal potential of some plant extracts against *Anopheles arabiensis* Patton (Diptera: Culicidae).Int J Trop Insect Sci. 2020; 41(2): 1-7. https://doi.org/10.1007/s42690-020-00229-1.

[40] EukubayA, GetuE, DebebeE, HadisM. Larvicidal activities of column chromatographic fractions of an ethanol leaf extract from *Ricinus communis* against *Anopheles arabiensis* Patton (Diptera: Culicidae).Ethiop JPubl Health Nutr. 2023; 6(1): 16-21.

[41] BoubaT, SaotoingP, NdjonkaD. Fractionation of methanolic extracts from two herbaceous plants and evaluation of their larvicidal activity on *Anopheles arabiensis*.J Trop Dis Publ Health. 2012; 9(1): 1-7.https://doi.org/10.35248/2329-891X.2021.9.263.

[42] YounoussaL, NukenineEN, EsimoneCO. Toxicity of *Boswellia dalzielii* (Burseraceae) leaf fractions against immature stages of *Anopheles gambiae* (Giles) and *Culex*

quinquefasciatus (Say) (Diptera: culicidae).Int J Insect Sci. 2016; 8: 23-31. https://doi.org/10.4137/IJIs.s37188.
[43] YounoussaL, NukenineEN, DangaYSP, AjaegbuEE, OsimoneCO.Laboratory evaluations of the fractions efficacy of *Annona senegalensis* (Annonaceae) leaf extract on immature stage development of malarial and filarial mosquito vectors.J Arthropod-Borne Dise. 2015; 9(2): 226-237. PMCID: PMC4662794.
[44] MoyesCL, VontasJ, MartinsAJ, NgLC, KoouSY, DusfourI. RaghavendraK, PintoJ, CorbelV, DavidJP, WeetmanD. Contemporary status of insecticide resistance in the major *Aedes* vectors of arboviruses infecting humans.PLoSNeglect Trop Dis. 2017;11(7): e0005625. https://doi.org/10.1371/journal.pntd.0005625.
[45] TanakaK. Studies on the metabolism, mode of action, and development of insecticides acting on the GABA receptor.JPesticid. 2019; 44(1): 71-86. https://doi.org/10.1584/jpestics.J18-04
[46] CostaMS, PaulaSO, MartinsGF, ZanuncioJC, SantanaAEG, SerrãoJE. Multiple modes of action of the squamocin in the midgut cells of *Aedes aegypti* larvae.PLoS ONE. 2016; 11(8):e0160928. https://doi.org/10.1371/journal.pone.0160928.
[47] CostaMS, PaulaSO, MartinsGF, ZanuncioJC, SantanaAEG, SerrãoJE. Modes of action of squamocin in the anal papillae of *Aedes aegypti* larvae.Physiol Mol Plant Pathol. 2018; 101: 172-177. https://doi.org/10.1016/j.pmpp.2017.04.001
[48] OteroALC, Vargas MéndezLY, DuqueLJE, KouznetsovVV. Design, synthesis, acetylcholinesterase inhibition and larvicidal activity of girgensohnine analogs on *Aedes aegypti*, vector of dengue fever.Europ J Med Chem. 2014;6(78) :392-400. https://doi.org/10.1016/j.ejmech.2014.03.067.
[49] PerumalsamyH, JangMJ, KimJ-R, KadarkaraiM, AhnY-J. Larvicidal activity and possible mode of action of four flavonoids and two fatty acids identified in *Millettia pinnata* seed toward three mosquito species.Parasites &Vect. 2015;8: 237-250. https://doi.org/10.1186/s13071-015-0848-8.
[50] Borrero-Landazabal MA, Carreño-Otero AL, Kouznetsov VV, Duque-Luna JE, Mendez-Sanchez SC, Landazabal MAB, Oterob ALC, Lunac JED. Alterations of mitochondrial electron transport chain and oxidative stress induced by alkaloid-like α-aminonitriles on *Aedes aegypti* larvae.PesticidBiochem Physiol. 2018; 144: 64-70. https://doi.org/10.1016/j.pestbp.2017.11.006.
[51] MaheswaranR, IgnacimuthuS.Effect of *Polygonum hydropiper* L. against dengue vector mosquito *Aedes albopictus* L.Parasitology Research. 2014; 113: 3143-3150. https://doi.org/10.1007/s00436-014-4037-z.
[52] MaheswaranR, IgnacimuthuS.Effect of confertifolin from Polygonum hydropiper L. against dengue vectormosquitoes*Aedes aegypti* L. Environ SciPollutRes. 2014: 1-8. https://doi.org/10.1007/s11356-020-10127-1.
[53] ManimegalaiT, RaguvaranK,KalpanaM, MaheswaranR.Green synthesis of silver nanoparticle using *Leonotisnepetifolia* and their toxicity against vector mosquitoes of *Aedes aegypti* and *Culex quinquefasciatus* and agricultural pests of *Spodoptera litura* and *Helicoverpaarmigera*.Environ Sci Pollut Res. 2014: 1-14.https://doi.org/10.1007/s11356-020-10127-1.

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