

Toxicity of the Methanol Extract and the Fractions of the Leaves of *Lippia adoensis* Hochst (Verbenaceae) against the Larvae of *Anopheles gambiae* Giles and *Culex quinquefasciatus* Say (Diptera: Culicidae)

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Abstract

Nowadays, the control of mosquitoes using phyto-insecticide products is strongly encouraged to the detriment of synthetic insecticides that are not biodegradable and are also toxic to humans and animals, as well as non-target living beings. The present study aimed to evaluate the efficacy of the methanolic crude extract of *Lippia adoensis* leaves and its fractions against *Anopheles gambiae* and *Culex quinquefasciatus* larvae under laboratory conditions. After the phytochemical analysis of each plant product, the methanolic crude extract and its five (5) fractions were diluted in 1 mL of methanol, and different concentrations of 125, 250, 500, and 1000 ppm were prepared in 100 mL of distilled water in 250 mL plastic cups. The commercial insecticide Bi-One (49% dichlorvos) was used as the positive control, while methanol (1 mL) was added to distilled water (99 mL) to constitute a negative control. Twenty-five (25) 4th instar larvae of each mosquito species were transferred into each prepared concentration solution and control. Mortality of the mosquito larvae was recorded 24 h post-treatment. Phytochemical analysis revealed the presence of alkaloids, phenolic compounds, terpenoids, flavonoids, tannins, and saponins in the methanolic extract and fractions of *L. adoensis*. Among the products tested, the methanolic crude extract was the most toxic to *An. gam-*

biae ($LC_{50} = 18.23$ ppm) and *Cx. quinquefasciatus* ($LC_{50} = 75.44$ ppm), followed by fraction 1 with CL_{50} values of 70.04 and 75.88 ppm on *An. gambiae* and *Cx. quinquefasciatus*, respectively. Due to their significant larvicidal activity, the methanolic crude extract and its fraction 1 might be considered as a promising candidate in the discovery of a novel botanical larvicide to reduce the mosquito larvae density.

Keywords

Larvicidal, Phytochemical, Extract, Fraction, *Lippia adoensis*, *Anopheles gambiae*, *Culex quinquefasciatus*

1. Introduction

Culicidae, or mosquitoes, are still considered today as potential vectors of diseases of medical importance in the world. These Culicidae include more than 3,500 species, mainly distributed in the tropical and subtropical regions [1]. They are always considered a source of nuisance for humans and also potential vectors in the transmission of several diseases. Females in the breeding season need blood for egg development, and some have a greater preference to feed on human blood [2]. Among these mosquito species, *Culex* and *Anopheles* genera are the best-known, responsible for the transmission of lymphatic filariasis and malaria, respectively [3].

Worldwide, 282 million cases and 610,000 deaths from malaria were reported in 2024 [4]. About 95% of cases (265 million), with 95% of deaths (579,000) from this disease, were reported in the WHO African region, in which about 75% occurred among children under 5 years old [4]. *Anopheles gambiae* s.l. is recognized as the main vector in this African region. In Cameroon, malaria remains the major endemic disease and the leading cause of morbidity and mortality, especially in children under five years and pregnant women [5]. In 2023, Cameroon was classified among the 15 countries with the highest burden of malaria cases, and from 2022 to 2023, the disease cases increased by 11.8% in this country [4].

Lymphatic filariasis, also known as elephantiasis, is a major vector-borne public health problem in the world. *Culex quinquefasciatus* is the main vector for the transmission of the human lymphatic worm, *Wuchereria bancrofti* [6]. About 120 million people in 72 countries were infected, with around 40 million people disabled by this disease in 2018 [7]. In Cameroon, lymphatic filariasis is endemic nationwide, with an estimated average of 3.3% of cases reported [8].

To deal with these diseases, several control methods have been developed, targeting the parasites and the vectors. Unfortunately, drug resistance in the treatment of malaria and a lack of specific drugs against lymphatic filariasis have been reported [9]. In addition, the cost of new antimalarial drugs is found to be very high on the market [10]. However, one of the methods that has demonstrated its effectiveness is the control of vectors (mosquito larvae and adults) through physical, biological, and chemical controls [11]. These vector control methods include the use of insect-

ticide-treated nets and indoor spraying of residual synthetic insecticides. Unfortunately, the repeated misuse of these synthetic chemicals has led to the development of mosquito strains resistant to these synthetic insecticides [12]. These chemicals are also harmful to humans and animals, and they pollute the environment due to their accumulation in nature [13]. In addition, their scarcity in local markets also leads to an increase in their costs [10]. Besides, these synthetic substances have a broad spectrum of action, not sparing non-target organisms [2].

Thus, it would be necessary to simultaneously research and improve the use of plant insecticides, which are well known by local communities and are biorational [14] [15]. About 2000 species of plants have proven to be effective against insects, with 344 species of plants exhibiting anti-mosquito activity [16].

Lippia adoensis (Verbenaceae) is an herbaceous plant comprising 41 genera with about 220 species and is widespread throughout tropical, western, and central Africa [17] [18]. This plant is used in traditional medicine to treat several diseases, such as bronchial inflammation, conjunctivitis, malaria, etc. [19]. Insecticidal efficacy of methanol extract and essential oils of its leaves against *Anopheles gambiae* has been reported by Oumarou *et al.* [20]. This plant has also been shown to be toxic against *Aedes* spp. and *An. arabiensis* [21] [22]. Nukenine *et al.* [23] reported the insecticidal efficacy of powder of leaves of *L. adoensis* against the pest of corn, *Sitophilus zeamais*. This study aimed to evaluate the toxicity of the leaf methanolic extract and fractions of *Lippia adoensis* against the larvae of the malarial vector *Anopheles gambiae* and lymphatic filariasis *Culex quinquefasciatus* in laboratory conditions.

2. Materials and Methods

2.1. Plant Material

2.1.1. Collection and Processing of Plant Material

The fresh leaves of *Lippia adoensis* were collected early in the morning, around 7 a.m. in Mbé (latitude 7°51.28'N, longitude 13°35.51'E, and altitude 601 m), located in the Vina Division, Adamaoua region of Cameroon, 78 km from Ngaoundere town. The plant specimen was identified and confirmed under registration N°10848/SRF.Cam in comparison with the botanical collection of F.J. Breteler N°826 at the National Herbarium of Cameroon.

The leaves were shade-dried in a room under ambient conditions ($27 \pm 2^\circ\text{C}$; $72 \pm 4\%$ r.h.) for 10 days. Then, the dried leaves were ground in a wooden mortar into a powder. This powder was sieved using a 0.4 mm mesh-size sieve, then packaged in dark plastic bags and stored in a refrigerator at -4°C until its use for extraction and fractionation.

2.1.2. Extraction and Fractionation

Extraction and fractionation of the leaf powders of *L. adoensis* were carried out in the Phytochemistry laboratory of the Institute of Medical Research and Medicinal Plants Studies (IMPM) of Yaounde. The initial extraction was carried out with

methanol solvent to obtain the residue called the methanolic crude extract. The choice of methanol as an initial solvent for extraction was based on its ability to extract several phytochemicals, especially those possessing insecticidal properties according to the literature. To obtain the methanolic crude extract, 1500 g of *L. adoensis* leaf powder were macerated in 6000 mL of methanol solvent using a percolator for 72 hours. The mixture was filtered using Whatman paper No.1, and the residue of maceration was rinsed and filtered several times with methanol until a clear phase was obtained. The filtrate was concentrated using a rotary evaporator to obtain a green, pasty extract called the methanolic extract. This extract was dried in an oven at 40°C.

For the fractionation process, 56 g of this methanolic crude extract of *L. adoensis* was fixed on 130 g of silica gel. After fixing, the powdery-looking mixture was transferred into a chromatographic column previously containing 364 g of pure silica and then eluted using a hexane solvent system (100%), followed by hexane-ethyl acetate with increasing polarities, then ethyl acetate (100%), then ethyl acetate-methanol (95%, 5%), and finally methanol (100%). At the end of column elution, 47 samples of 500 mL fractions were collected, then concentrated using a rotary evaporator and grouped into five major fractions (1 - 14 (fraction 1), 15 - 19 (fraction 2), 20 - 33 (fraction 3), 34 - 44 (fraction 4), 45 - 47 (fraction 5)) using analytical thin layer chromatography (TLC). The crude extract and the fractions were stored at -4°C in a refrigerator until used for screening phytochemicals and bioassays. The yields of *L. adoensis* methanolic extract and its fractions were obtained and calculated according to the formula below.

$$\text{Yield}(\%) = \frac{\text{Weight of extract or fraction obtained}}{\text{Weight of plant powder or methanolic crude extract used}} \times 100$$

2.1.3. Phytochemical Screening of Plant Products

The qualitative phytochemical screenings of some compound groups contained in *L. adoensis* extract and its fractions were carried out according to the method of Harborne [24] (1998). The plant methanolic extract and its fractions were subjected to phytochemical analysis to detect the presence of alkaloids, phenolic compounds, terpenoids, steroids, flavonoids, tannins, and saponins, which, according to the literature, possess insecticidal properties.

2.2. Strains of Mosquito Species Used

The eggs of the laboratory-maintained strain of *An. gambiae* (insecticide-susceptible strain) were provided by the Coordination Organization for the Control against Endemics in Central Africa (OCEAC) in Yaounde, while rafts of *Cx. quinquefasciatus* were collected from the strain established in the insectarium of the Laboratory of Applied Zoology, Faculty of Science, University of Ngaoundere, Cameroon. In the insectarium, each mosquito egg was transferred into trays containing tap water to hatch into larvae. Each mosquito species' larvae were reared following the procedure described by the WHO protocol [25] under laboratory conditions (25 ± 2°C;

74 ± 4% RH). Mosquito larvae were fed with a diet containing crayfish and biscuit in a ratio of 1:3. The water in each tray was renewed every two days to avoid the suffocation of mosquito larvae caused by decayed food. Fourth instar larvae of *An. gambiae* and *Cx. quinquefasciatus* were used for the larvicidal test.

Toxicity of *Lippia adoensis* Extract and Its Fractions on *An. gambiae* and *Cx. quinquefasciatus* Larvae

Larvicidal activity of methanolic crude extract and fractions of *L. adoensis* was evaluated on the fourth instar larvae of *An. gambiae* and *Cx. quinquefasciatus* according to the method described by WHO [25]. The plant extract and its fractions were dissolved in 1 mL of methanol, and concentrations of 125, 250, 500, and 1000 ppm were prepared in a volume of 100 mL with distilled water in 250 mL beakers. The negative control consisted of adding 1 mL of methanol to 99 mL of distilled water. Bi-One (49% dichlorvos) was used at the recommended concentration of 1000 ppm as a positive control. Twenty-five (25) fourth instar larvae of each mosquito species, randomly collected, were transferred into each prepared concentration solution and controls. Three replicates were maintained for each concentration, and larval mortality was recorded after 24 hours. The mortality rate was calculated according to the formula below:

$$\text{Mortality (\%)} = \frac{\text{Number of dead larvae}}{\text{Total number of larvae used}} \times 100$$

2.3. Statistical Analysis

Abbott’s formula [26] was applied for mortality correction whenever required before probit analysis and ANOVA. The percentage of mortality data was subjected to analysis of variance (ANOVA) using Statistical Package for the Social Sciences (SPSS 16.0). Tukey’s test (P = 0.05) was applied for means comparison. The probit analysis [27] was applied to determine lethal concentrations causing 50% (LC₅₀) and 90% (LC₉₀) mortality of larvae 24 hours post-treatment.

3. Results

3.1. Extraction and Fractionation Yield of *L. adoensis*

Results of extraction and fractionation yields are presented in **Table 1**. Extraction of 1500 g of *L. adoensis* leaf powder in 6000 mL of methanol yielded 10.26% of methanolic crude extract. The fractionation of 56 g of the methanolic crude extract allowed the obtainment of five major fractions with yields of 23.25, 9.89, 1.91, 17.10, and 42.71% for fractions 1, 2, 3, 4, and 5, respectively (**Table 1**).

Table 1. Yields of extraction and fractionation of *Lippia adoensis* leaves.

Extract/Fraction	Solvent systems with increasing polarities	Yields (%)
Crude extract	Methanol (100%)	10.26
Fraction 1	Hexane/ethyl acetate (100:0; 95:5; 85:15)	23.25

Continued

Faction 2	Hexane/ethyl acetate (70:30)	9.89
Fraction 3	Hexane/ethyl acetate (70:30; 50:50)	1.91
Fraction 4	Ethyl acetate/methanol (100:0; 95:5)	17.10
Fraction 5	Ethyl acetate/methanol (0:100)	42.71

3.2. Phytochemical Sreening

Table 2 presents some phytochemical components of the extract and fractions of *L. adoensis*. The methanolic crude extract revealed the presence of all the compounds tested, namely alkaloids, phenolic compounds, terpenoids, steroids, flavonoids, tannins, and saponins. After fractionation, the compounds were distributed into different fractions obtained according to their polarity. Phenolic compounds, steroids, tannins, and saponins were found in fraction 1, while alkaloids, phenolic compounds, terpenoids, and steroids were present in fraction 2. Almost all compounds tested were found in fraction 3, except for flavonoids and saponins, which were absent. In fraction 4, alkaloids, phenolic compounds, terpenoids, and steroids were present. Fraction 5 revealed the presence of all compounds except alkaloids and tannins, which were absent.

Table 2. Some phytochemicals in the extract and fractions of leaves of *Lippia adoensis* after a qualitative phytochemical test.

Phytochemicals	Crude extract	Fraction 1	Fraction 2	Fraction 3	Fraction 4	Fraction 5
Alkaloids	+	–	+	+	+	–
Phenolic compounds	+	+	+	+	+	+
Terpenoids	+	–	+	+	+	+
Steroids	+	+	+	+	+	+
Flavonoids	+	–	–	–	–	+
Tannins	+	+	–	+	–	–
Saponins	+	+	–	–	–	+

– = absent; + = present.

3.3. Toxicity of *Lippia adoensis* Extract and Fractions on *Anopheles gambiae* Larvae

Figure 1 shows the mortality rates of *An. gambiae* larvae treated with leaf methanolic crude extract and fractions of *L. adoensis* under laboratory conditions. Treated with the methanolic extract and fractions of *L. adoensis*, the mortality rate of *An. gambiae* after 24 h post-exposure significantly increased with increasing concentrations and varied depending on the fraction or extract applied.

All the plant products tested caused significant mortality ($F_{(5,12)} = 339.71$; $P < 0.001$) of *An. gambiae* larvae, varying from 0.00% to 93.33% at the smallest concentration (125 ppm) and from 56.66% to 100% ($F_{(5,12)} = 1881.46$; $p < 0.001$) at the highest concentration (1000 ppm). The methanolic crude extract caused highly

significant mortality ($F_{(5,12)} = 785.71$; $p < 0.001$), varying from 93.33% at the small concentration (125 ppm) to 100% at the concentration of 250 ppm, 24 h post-exposure (**Figure 1**). Treated at a concentration of 125 ppm, mortalities of 77.33% and 45.33% were recorded, respectively, with fractions 1 and 2 after 24 hours of exposure. For fractions 1 and 2, larval mortality reached 100% at the concentration of 500 ppm after 24 hours of exposure. Treated at 125 ppm, 53.33% and 5.33% mortalities were recorded after 24 hours of exposure for fractions 3 and 4, respectively, while no larval mortality was recorded in fraction 5 after 24 hours of exposure. Treated at the highest concentration (1000 ppm) with these same fractions, 97.33%, 86.66%, and 54.66% larval mortality rates were recorded, respectively, with fractions 3, 4, and 5 after 24 h of exposure. With the positive control (Bi-One 1000 ppm), 100% larval mortality was observed after 24 h of exposure, while with the negative control, no larval mortality was recorded.

The values of lethal concentrations killing 50 and 90% of the larvae are presented in **Table 3**. These values varied from one fraction to another after 24 h of exposure. The methanolic crude extract was the most potent larvicide, with the lowest value of LC_{50} of 18.23 ppm and LC_{90} of 85.65 ppm, followed by fraction 1 ($LC_{50} = 70.04$ ppm and $LC_{90} = 205.29$ ppm), fraction 3 ($LC_{50} = 102.10$ ppm and $LC_{90} = 293.79$ ppm), and fraction 2 ($LC_{50} = 138.84$ ppm and $LC_{90} = 531.88$ ppm). Fractions 4 ($LC_{50} = 400.40$ ppm and $LC_{90} = 1152$ ppm) and fraction 5 ($LC_{50} = 909.35$ ppm and $LC_{90} = 2399$ ppm) were less effective after 24 h of exposure against *An. gambiae* larvae.

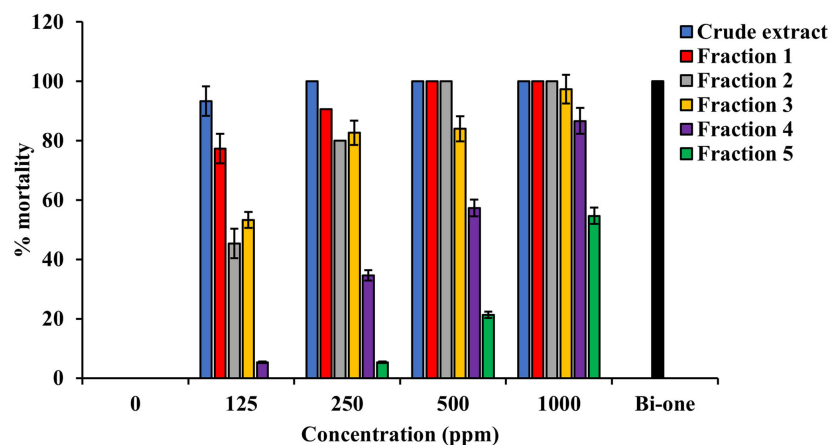


Figure 1. Mortality rate of *An. gambiae* larvae after 24 hours post-treatment with the methanolic crude extract and fractions of *Lippia adoensis* under laboratory conditions ($25 \pm 2^\circ\text{C}$; $74 \pm 4\%$ RH).

Table 3. LC_{50} and LC_{90} (ppm) values of methanolic crude extract and fractions of *Lippia adoensis* after 24 hours post-exposure against *Anopheles gambiae* larvae under laboratory conditions ($25 \pm 2^\circ\text{C}$; $74 \pm 4\%$ RH).

Extract/Fractions	Slope $\pm$ SE	R^2	LC_{50} (FL)	LC_{90} (FL)	χ^2
Crude extract	1.90 ± 0.46	0.48	18.23 (0.008 - 49.97)	85.65 (7.42 - 130.16)	20.63*
Fraction 1	2.74 ± 0.30	0.78	70.04 (52.90 - 84.59)	205.29 (185.40 - 230.95)	10.07 ^{ns}

Continued

Fraction 2	3.93 ± 0.27	0.76	138.84 (121.29 - 154.43)	531.88 (401.24 - 853.70)	20.70*
Fraction 3	1.78 ± 0.14	0.81	102.10 (57.46 - 140.79)	293.79 (258.58 - 351.36)	35.93***
Fraction 4	2.79 ± 0.14	0.95	400.40 (374.10 - 428.99)	1152 (1022.17 - 1328.18)	13.23 ^{ns}
Fraction 5	3.04 ± 0.20	0.99	909.35 (835.18 - 1004.77)	2399 (2013.93 - 3000.46)	9.34 ^{ns}

*** $P < 0.001$; * $P < 0.05$; ^{ns} $P > 0.05$; FL = Fiducial Limit; LC = Lethal Concentration; R^2 = coefficient of determination; χ^2 = Chi-square.

3.4. Efficacy of *Lippia adoensis* Extract/Fractions against *Culex quinquefasciatus* Larvae

The percentages of mortality of *Cx. quinquefasciatus* larvae treated with different doses of *L. adoensis* methanolic crude extract and its five fractions are presented in Figure 2. In general, larval mortality increased with increasing concentrations and varied from one fraction to another after 24 h post-exposure.

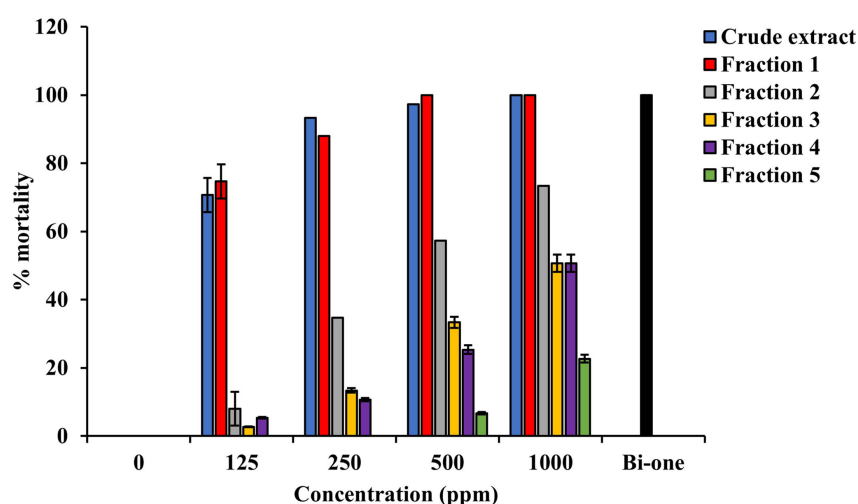


Figure 2. Mortality percentage of *Culex quinquefasciatus* larvae after 24 hours post-treatment with *Lippia adoensis* methanolic crude extract and fractions under laboratory conditions ($25 \pm 2^\circ\text{C}$; $74 \pm 4\%$ RH).

At 125 ppm, all the 5 fractions and the methanolic crude extract induced significant larval mortality ($F_{(5,12)} = 610.42$; $P < 0.001$), varying from 0.00% in fraction 5 to 70.66% in the methanolic crude extract after 24 h post-exposure. The methanolic crude extract caused high larval mortality, varying significantly ($F_{(5,12)} = 1734$; $p < 0.001$) from 70.66% at the lowest concentration (125 ppm) to 100% at the highest concentration (1000 ppm). Likewise, high larval mortality was observed with fraction 1, ranging significantly ($F_{(5,12)} = 1290$; $p < 0.001$) from 74.66% at the lowest concentration (125 ppm) to 100% from a concentration of 500 ppm after 24 h post-exposure. Fraction 2 also exhibited significant larval mortality ($F_{(5,12)} = 841.36$; $p < 0.001$), varying from 8.00% at 125 ppm to 73.33% at 1000 ppm. At 125 ppm, mortality rates of 2.66% and 5.33% were recorded with fractions 3 and 4, respectively, after 24 h post-exposure, while no larval mortality was recorded with fraction 5 at the same exposure period. Treated at the highest concen-

tration (1000 ppm), a mortality rate of 50.66% was recorded with both fraction 3 and fraction 4 after 24 h post-exposure, while with fraction 5, a mortality of 22.66% was recorded at the same exposure period (**Figure 2**). Total larval mortality (100%) was recorded in the positive control (Bi-One 1000 ppm); no larval mortality was recorded in the negative control after 24 h post-exposure.

The values of lethal concentrations killing 50 and 90% of *Cx. quinquefasciatus* larvae are presented in **Table 4**. These values varied from one fraction to another. The methanolic extract and fraction 1 were the most effective larvicides with LC₅₀ values of 75.44 and 75.88 ppm and LC₉₀ values of 232.86 and 225.37 ppm, respectively, after 24 hours of exposure. Fractions 2, 3, 4, and 5 were the least effective, with LC₅₀ values of 448.18, 908.74, 1040, and 1718 ppm, respectively, after 24 hours of exposure (**Table 4**).

Table 4. LC₅₀ and LC₉₀ (ppm) values of the methanolic crude extract and fractions of *Lippia adoensis* after 24 hours post-exposure against *Culex quinquefasciatus* larvae under laboratory conditions (25 ± 2 °C; 74 ± 4% RH).

Extract/Fractions	Slope ± SE	R ²	LC ₅₀ (FL)	LC ₉₀ (FL)	χ ²
Crude extract	2.61 ± 0.25	0.73	75.44 (59.26 - 89.62)	232.86 (210.16 - 262.52)	13.20*
Fraction 1	2.71 ± 0.28	0.79	75.88 (50.68 - 96.08)	225.37 (194.97 - 271.84)	16.62 ^{ns}
Fraction 2	2.12 ± 0.12	0.91	448.14 (396.08 - 511.47)	1797 (1396.92 - 2537.97)	16.51 ^{ns}
Fraction 3	2.04 ± 0.14	0.97	908.74 (805.97 - 1051.05)	3855 (2941.60 - 5488.14)	8.80 ^{ns}
Fraction 4	1.89 ± 0.14	0.99	1040 (903.20 - 1239.42)	4933 (3595.39 - 7508.79)	7.55 ^{ns}
Fraction 5	3.04 ± 0.35	0.96	1718 (1441.36 - 2239.56)	4521 (3208.63 - 7746.03)	7.92 ^{ns}

*P < 0.05; ^{ns}P > 0.05; FL = Fiducial Limit; LC = Lethal Concentration; R² = coefficient of determination; χ² = Khi-squared.

4. Discussion

Eliminating mosquitoes at the larval stage before they reach the stage at which they can transmit diseases would seem to be an ideal and suitable approach for mosquito control. However, plants have been reported to be useful to control mosquitoes. In the present study, a significant concentration-dependent larvicidal activity was demonstrated with the extract and fractions of *L. adoensis* against the fourth instar larvae of *An. gambiae* and *Cx. quinquefasciatus*.

The cold extraction process of the leaves of *L. adoensis* with methanol solvent gave a yield of 10.26%. The result obtained in this present study is likely comparable to the yields obtained by Oumarou *et al.* [20], in which a yield of 9.76% of the methanol extract of *L. adoensis* harvested at Mbe (Adamawa region, Cameroon) was recorded. This result is also similar to those obtained by Younoussa [28] with the seeds of *Dacryodes edulis* (9.41%) and *Mangifera indica* (11.32%). On the contrary, the yield obtained by Danga *et al.* [29] with the leaves of *Callistemon rigidus* (27.28%) extracted through the same process and the same solvent was higher than those registered in the present study. The same tendency was observed in the extraction yield of 15.9% obtained by Abdoulaye *et al.* [30] with the leaves of *Eucalyptus camaldulensis* using methanol solvent. These differences in plant extrac-

tion yields could be due to the harvest period of the plant, the part of the plant used, the species of plant, the extraction method and procedure, the extraction time, the chemical composition of the plant, as well as the polarity of the solvents used [31].

After the fractionation of *L. adoensis* methanolic crude extract, the results showed high fractionation yields with polar solvents compared to non-polar solvents used for column elution. Similar results were reported by Younoussa *et al.* [28], in which the fraction yield increased with the polarity of the solvent used, ranging from 6.45% with nonpolar (n-hexane) to 68.24% with the polar solvent (methanol). These results are also in agreement with those of Sultana *et al.* [31] and Shabbir *et al.* [32], reporting that plant components are often extracted in larger quantities with polar solvents such as water, methanol, or ethanol.

The seven phytochemical constituents screened in the present study, including alkaloids, phenolic compounds, terpenoids, steroids, flavonoids, tannins, and saponins, were present in the methanolic crude extract of *L. adoensis*. Similar results were reported by Oumarou *et al.* [20] in the methanolic extract of *L. adoensis* collected from Mbé (Adamawa region, Cameroon). Likewise, Abdoulaye *et al.* [30] reported the presence of alkaloids, terpenoids, tannins, saponins, flavonoids, and phenolic compounds in the methanol extract of leaves of *E. camaldulensis*. The qualitative variations in phytochemical components across and within plant species could be attributed to seasonal and maturity variation, geographical origin, growth stages, part of the plant used, postharvest drying, and storage [33] [34]. After the fractionation of this same methanolic crude extract of *L. adoensis*, these phytochemical components were distributed into the different fractions obtained according to the ability of each solvent to extract the phytochemical components following their polarity.

Regarding the larvicidal activity of the products tested, all the fractions and the methanolic crude extract of *L. adoensis* leaves exhibited significant, concentration-dependent larvicidal activity against fourth instar larvae of *An. gambiae* and *Cx. quinquefasciatus*.

However, in this present study, the crude methanolic extract of *L. adoensis* was the most toxic to *An. gambiae* and *Cx. quinquefasciatus* compared to its fractions. Similarly, the larvicidal and repellent activities of *Eucalyptus* and *Ocimum* species extracts against *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* were higher than their fractions [35] [36]. A similar observation was also reported with *Lantana camara*, *Azadirachta indica*, and *Ocimum gratissimum*, in which strong insecticidal and repellent activities against *Spodoptera litura* and *Sitophilus zeamais* were registered with the plant crude extracts compared to their fractions or isolated compounds [37] [38].

In fact, since plant extracts possess diverse phytoconstituents, the minor compound classes may enhance the toxicity or penetration of the major active compounds in the insect, leading to higher mortality than fractions [39] [40]. Besides, the solubility and stability of active compounds contained in the plant extracts are

improved by certain phytochemicals, which may be lost during the fractionation process [39]. According to Regnault-Roger *et al.* [38], the plant extract may act with multi-target action (nervous system function, respiratory processes, growth, and development), leading to higher mortality of mosquito larvae compared to its fractions that may act on one target.

Among the fractions obtained by splitting the methanolic crude extract of *L. adoensis*, fraction 1 was revealed as the most potent on *An. gambiae* and *Cx. quinquefasciatus* larvae. In the same way, fraction 1 of *Chenopodium ambrosoides* was reported to be the most potent against both *An. gambiae* (CL₅₀ = 66.39 ppm) and *Cx. quinquefasciatus* (CL₅₀ = 251.41 ppm) larvae compared to the other fractions [41]. Since fraction 1 was entirely eluted with hexane solvent, it is considered the hexane fraction. The effectiveness of fraction 1 is comparable to the results by Danga *et al.* [42] in which the n-hexane fraction of *Callistemon rigidus* was more effective on *Aedes aegypti*, *An. gambiae* and *Cx. quinquefasciatus* larvae than chloroform, ethyl acetate, and methanol fractions of the plant. Azokou *et al.* [43] also reported that the hexane fractions of *Cissus populnea*, *Cochlospermum planchonii*, and *Phyllanthus amarus* showed high larvicidal activity against resistant and sensitive larvae (III and IV instar) of *An. gambiae* and *Cx. quinquefasciatus*. The effectiveness of the hexane fraction in this study could be linked to the presence of oil, which could block spiracles, resulting in asphyxiation and death of the larvae [42]. The efficacy of the n-hexane fractions was supported by Anupam *et al.* [44] when they reported that n-hexane, the most non-polar, mainly extracts essential oil recognized as a powerful insecticide.

In this study, the methanolic crude extract and fractions of *L. adoensis* acted at different levels of effectiveness on *An. gambiae* and *Cx. quinquefasciatus* larvae. The efficacy of methanolic crude extract and fractions could be due to various compounds, including phenolics, alkaloids, terpenoids, flavonoids, steroids, saponins, and tannins acting jointly or independently on mosquito larvae [45]. Indeed, previous studies have demonstrated that phytochemicals interfere with the proper functioning of mitochondria, especially at the proton-transferring sites [46]. However, the secondary metabolites found in the methanolic extract and the fractions cause physiological and cellular disturbances that include inhibition of acetylcholinesterase, disruption of sodium and potassium ion exchange, and interference with mitochondrial respiration [46]. Additionally, they affect the mid-gut epithelium or gastric caecae and the malpighian tubes in mosquito larvae [47].

According to Faraway [48], in a regression analysis model using result data from biological experiments, the results are favorably attributed to the efficacy of the products tested in the case of $R^2 \geq 0.6$. But in this present investigation, most of the R^2 values of *L. adoensis* extract and fractions against larvae of *An. gambiae* and *Cx. quinquefasciatus* were ≥ 0.6 . Some coefficient of determination values less than 0.6 encountered could be attributed to the high doses of products applied, which could lead to almost or complete activity without variation in responses. Besides, the majority of the chi-square (χ^2) values of the plant extract and fractions

were not significant, implying an approximation of the regression models to the theoretical models concerning the effectiveness of the products against mosquito species.

5. Conclusion

The methanolic crude extract of *L. adoensis* and its 5 fractions exhibited significant larvicidal activity against the main vectors of malaria (*An. gambiae*) and lymphatic filariasis (*Cx. quinquefasciatus*). Indeed, the methanolic crude extract of *L. adoensis* yielded 10.26%, while fraction 5 (42.71%) yielded more than the other fractions. All seven classes of phytochemicals (alkaloids, terpenoids, flavonoids, saponins, tannins, phenolic compounds, and steroids) targeted were present in the methanolic crude extract and were relatively distributed within the 5 fractions of the plant. Globally, larvae of *An. gambiae* were more susceptible to all the plant products than *Cx. quinquefasciatus* larvae. Compared to the fractions of *L. adoensis*, the methanolic crude extract of the plant was the most active on the 2 mosquito species' larvae, with LC₅₀ values of 18.23 ppm against *An. gambiae* and 75.44 ppm against *Cx. quinquefasciatus* larvae. Thus, the methanolic crude extract of *L. adoensis* might be considered a promising candidate for the development of a new botanical larvicide to control *An. gambiae* and *Cx. quinquefasciatus* larvae in their breeding sites around human dwellings. The current data support laboratory larvicidal activity for these mosquito species after 24 h exposure, but they do not yet address non-target safety, persistence, or field performance.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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