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Qualitative and quantitative analysis of the essential oil extracted from Leucas Martinicensis and larvicidal effect on Anopheles Gambiae Giles 1902

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***Abstract*---Objectives:** The present work aimed to conduct a qualitative and quantitative analysis of the essential oil extracted from *Leucas Martinicensis* and to evaluate its larvicidal effect on *Anopheles gambiae* Giles 1902. **Methods:** The plant used in this work and identified at the herbarium of the Garoua School of Wildlife under number HEFG1435 was collected in Maroua in August 2023 and then

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cleaned of debris. The essential oil was obtained through hydrodistillation using a Clevenger-type apparatus. Phytochemical analysis was carried out using standard qualitative and quantitative methods. The tests involved evaluating the mortality of stage II and IV larvae of *Anopheles gambiae* ss (sensu stricto) in the presence of diluted solutions of the essential oil of *Leucas martinicensis* following a methodology of the WHO (2005). **Results:** Qualitative and quantitative analyses of the essential oil of *Leucas martinicensis* revealed the presence of various phytochemical constituents such as polyphenols (17.87±0.44g equivalent gallic acid/100g DM), terpenoids (30.62±0.6g equivalent lupeol/100g DM), flavonoids (13.61±0.38g equivalent quercetin/100g DM), tannins (8.83±0.23g equivalent catechin/100g DM), and saponins (5.94±0.51g equivalent galactose/100g DM) in the essential oil of *Leucas martinicensis*. The results of biological tests showed strong larvicidal activity against *Anopheles gambiae* sensu stricto. Indeed, the essential oil of *Leucas martinicensis* killed 100% of the larvae at the following concentrations and times: 50ppm in 24 hours, 100ppm in 12 hours, 150ppm in 8 hours, and 200ppm and 250ppm in 6 hours. The observed insecticidal potential can be attributed to the presence of phenolic, terpenic, and many other bioactive compound. **Conclusion:** This study highlights the phytochemical diversity and larvicidal potential of the essential oil extracted from *Leucas Martinicensis*. The presence of various phytochemicals suggests its potential as a natural source of bioactive compounds. Moreover, the significant larvicidal activity observed from the essential oil indicates its possible application in the phytopharmaceutical industries.

***Keywords*---**Qualitative, quantitative analysis, essential oil, *Leucas martinicensis*, larvicidal effect, *Anopheles gambiae*.

1. Introduction

Nowadays, malaria has become a public health problem despite the efforts made by various countries around the world. According to the WHO report (2024), the number of malaria cases recorded worldwide in 2023 is estimated at 263 million people, with the number of deaths at 597,000 (WHO, 2024). Africa, a continent where many at-risk individuals still do not have access to the necessary services to prevent, detect, and treat this disease, unfortunately remains the most affected, with about 95% of deaths (WHO, 2024). Malaria is a febrile condition caused by a sporozoan of the genus *Plasmodium* spp, transmitted from an infected individual to another by the Anopheles mosquito, an anthropophilic vector that is very abundant, especially in urban areas where larvae develop in collections of clear water and whose females preferentially bite at night inside buildings (Carnevale, 2009). The use of larvicides remains one of the best vector control methods, particularly for those of the *Anopheles* genus (Kiran *et al.*, 2006). In recent years, vector control has faced numerous obstacles related to the use of synthetic chemical insecticides that induce resistance among vectors (Akono-Ntonga *et al.*, 2016). Today, many studies highlight the insecticidal properties of

plants derived from traditional African pharmacopoeia, revealing that the insecticidal potential of these plants is enhanced in the form of essential oils (Saotoing, 2017). The number of bio-active molecules found in these volatile essences and their ability to diffuse through the integuments of arthropods are thought to be the reason for this effectiveness (Lucia *et al.*, 2007). Cameroon is rich in a strong floral diversity with invaluable therapeutic, insecticidal, and insect-repellent resources (Saotoing, 2017). However, most of these properties are still underused or exploited in a traditional way (Bouba, 2022). This is the case of *Leucas martinicensis*, whose leaves are traditionally used in some African regions as an insect repellent. Originating from South America and the Antilles, *Leucas martinicensis* is an annual, aromatic plant that grows up to 1.5m tall and is found in grassy areas, wasteland near dwellings (Hyde and Wursten, 2009). It is a plant widely distributed in the tropical regions of Africa, Arabia, Asia, and America (Muhammad *et al.*, 2012). The genus *Leucas* encompasses a diverse group of species known for their bioactive compounds and their insecticidal and pharmaceutical potential (Musa, 2017). Among these, *Leucas martinicensis* has drawn attention as an insecticidal plant due to its traditional use by populations. Exploring the phytochemical composition of *Leucas martinicensis* can provide valuable information about its larvicidal potential and contribute to its use in various health-related applications (Mohammed *et al.*, 2012), including the fight against malaria through the reduction of vector populations of Plasmodium spp. This research aims to conduct a qualitative and quantitative analysis of the essential oil extracted from *Leucas martinicensis* and to evaluate its larvicidal effect on *Anopheles gambiae* ss.

2. Materials and Methods

2.1. Collection of plant material

Leucas martinicensis (Figure 1) was collected in Maroua in August 2023, and then identified at the herbarium of the School of Wildlife in Garoua under the numbers HEFG1435.

Figure 1: *Leucas martinicensis*

2.2. Extraction of essential oil from *Leucas martinicensis*

The essential oil of *Leucas Martinicensis* was obtained by steam distillation using a steam distiller with a Clevenger apparatus (Kubmarawa *et al.*, 2013). Indeed, a mass of 500g of plant material was subjected to hydrodistillation for 2 hours in a pot in the presence of 3L of water. After boiling, sodium chloride and hexane were added to the hydrosol, and the mixture obtained was then introduced into a separating funnel (Runde *et al.*, 2015). The oil, being less dense than the water that floats on top, is collected from the tap, filtered, and then placed in the rotavapor to evaporate the hexane. The essential oil obtained was kept cold at -4 °C until larvicidal tests. The extraction steps are shown in Figure 2.

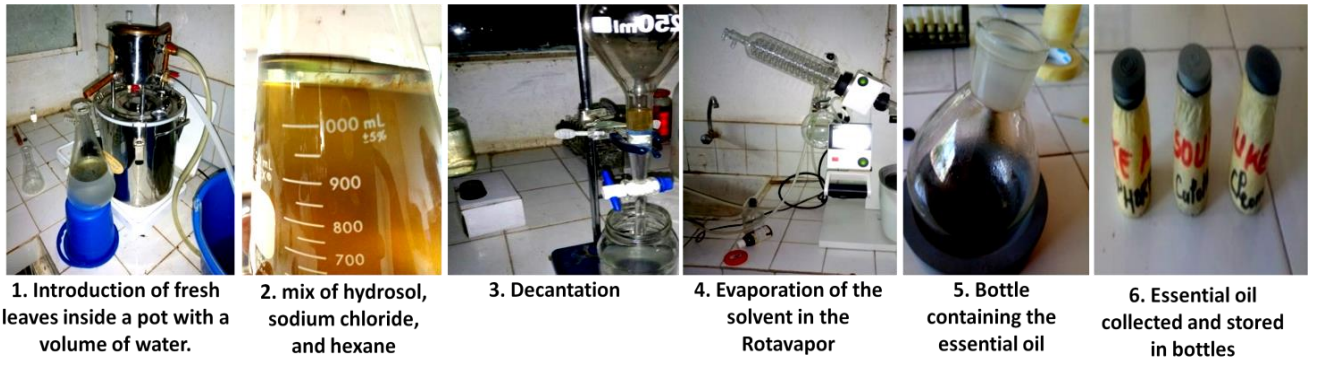


Figure 2: Steps for the extraction of essential oil from *Leucas martinicensis*

2.3. Phytochemical analyses

The phytochemical analysis of the essential oil was performed using standard qualitative methods as described by Kubmarawa *et al.* (2013) and Kadda *et al.* (2022).

2.3.1. Qualitative analyses

Phytochemical screening is a qualitative analysis based on precipitation and/or coloration reactions. These allow for the determination of the presence or absence of secondary metabolites such as polyphenols, flavonoids, saponins, tannins, terpenoids, etc., in the various organs of the plant (Singleton *et al.*, 1999).

2.3.2. Quantitative Analyses

A quantity of 1mL of essential oil of *Leucas martinicensis* was introduced into 10 mL of 80% methanol. The resulting mixture was diluted 50 times, followed by the quantification of total polyphenols, flavonoids, saponins, tannins, and terpenoids.

2.4. Filling with water of *Anopheles gambiae* Giles 1902 eggs and obtaining the larvae

The eggs of *Anopheles gambiae* ss were provided by the Organization for the Coordination of Endemic Diseases in Central Africa (OCEAC) based in Yaounde-Cameroon with the aim of being maintained in breeding to obtain larvae for larvicidal tests. The breeding was carried out at the Entomology laboratory of the University of Ngaoundere in October 2023. The eggs were soaked in plastic trays containing untreated natural well water. A few hours (18h – 24h) after soaking the eggs, they hatched into stage I larvae which visibly measure 2 mm in length. The stage I larvae developed into stage II larvae after 48 hours. After 4 days, the larvae

had reached stage IV. The larvae were fed with Tetrababyfishfood (Desfontaine *et al.*, 1991). The stage II and IV larvae that made up the larvicidal test population were each 24 hours old.

2.5. Evaluation of larvicidal activity

The tests consisted of evaluating the mortality of *Anopheles gambiae* ss larvae in the presence of diluted solutions of essential oil extracted from *Leucas martinicensis* following a methodology inspired by the WHO protocol (2005). For this, ten (10) batches of 25 larvae (comprising 5 batches of stage II larvae and 5 batches of stage IV larvae) were collected using a pipette and placed in 10 small transparent plastic boxes measuring 10 x 6 x 3.6 cm, each containing a volume of the stock solution (diluted essential oil) supplemented with a volume of breeding water up to 100 mL, total volume. Four control boxes with different contents were prepared: two positive control boxes (stage II and stage IV) containing temephos and two negative control boxes (stage II and stage IV) containing hexane. The experimental device with all 14 boxes is presented in Table I. The cumulative counting of dead larvae was done in hourly intervals every 2 hours during 12 hours of exposure to different concentrations of extracts, and the setup is maintained until 24 hours, at which time mortality was evaluated. A larva is considered dead when it remains immobilized at the bottom of the box and does not react to touch with a needle.

Table I: Experimental device

Concentration (ppm)	150	300	500	750	1000	Positive witness (Temephos)	Negative witness (Hexane)
Volume of stock solution to be taken (ml)	0,15	0,3	0,5	0,75	1		
Number of larvae	25	25	25	25	25	25	25
Stage II							
Stage IV							

2.6. Data analysis

The data were entered and analyzed using R software. Chi-squared (x2) homogeneity tests were performed to compare the observed mortality rates. Pearson's R correlation test was used to calculate the strength of the relationship between the corresponding LC_{50} and LH_{50}. Excel 2007 was used to plot curves and regression lines. Finney's (1971) formulas were used to calculate LC_{50} and LH_{50}.

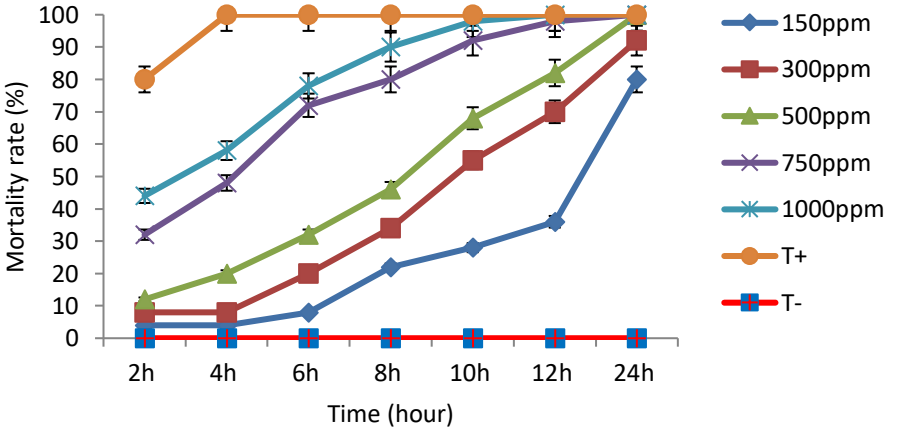
$$LC_{50}(LH_{50}) = log10^{-1}\left(\frac{y}{a} - \frac{b}{a}\right)$$

For all analyses, results with: $p<0.05$ are significant

3. Results

3.1. Effect of essential oils on stage II larvae

The evaluation of the mortality rates of *Anopheles gambiae* ss larvae exposed to different doses of essential oil from *Leucas martinicensis* yielded results presented in Figure 3. The concentrations of 150ppm and 250ppm became active from 2 hours of exposure, inducing a very low mortality rate of 4% and 8%, which increased after 24 hours to 80% and 92%. A similar trend was observed for the doses of 750ppm and 1000ppm, which killed 100% of the larvae after 24 hours of exposure. A significant difference (x^2= 49.99; *df* = 4) was observed between these mortality rates.

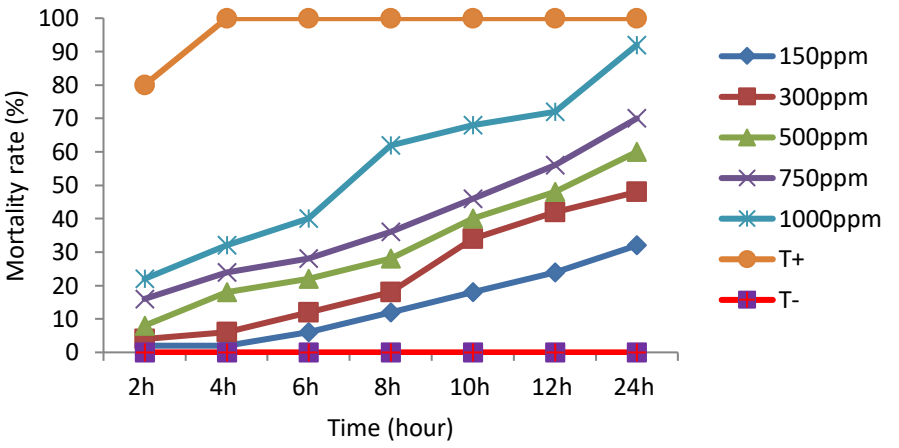


T- : negative control (hexane); T+ : positive control (temephos)

Figure 3: Mortality rate of stage II larvae to the essential oil of *Leucas martinicensis*

3.2. Effect of essential oils on stage IV larvae

The evolution of mortality rates for stage IV larvae of *Anopheles gambiae* ss represented in Figure 4 shows that not all concentrations induced 100% mortality after 24 hours of exposure to the essential oil of *Leucas martinicensis*. Indeed, the different concentrations of 150ppm and 300ppm, which caused 2% and 4% mortality respectively at the 2nd hour of exposure, killed 32% and 48% of the larvae after 24 hours. The other concentrations of 500ppm, 750ppm, and 1000ppm, which started their larvicidal activity at the 2nd hour of exposure with 8%, 16%, and 22% mortality respectively, killed 60%, 70%, and 92% of the larvae after 24 hours of exposure respectively. A significant difference was noted between the mortality rates (X2 = 38.653, *df* = 4, p-value = 0.02968).



T- : negative control (hexane); T+ : positive control (temephos)

Figure 4: Mortality rate of stage IV larvae to the essential oil of *Leucas martinicensis*

3.3. LC_{50} and LH_{50} of essential oil on stage II and IV larvae

3.3.1. Regression lines for the determination of LC_{50} of essential oil from *L. martinicensis*

The determination of the lethal concentration 50 (LC_{50}) of essential oil extracted from *Leucas martinicensis* on stage II and IV larvae of *Anopheles gambiae* ss was carried out according to the Finney method (1971) based on the regression lines (Figure 5) obtained by transforming the percentages of mortality into probit after 24 hours of exposure based on the decimal logarithm of the concentrations.

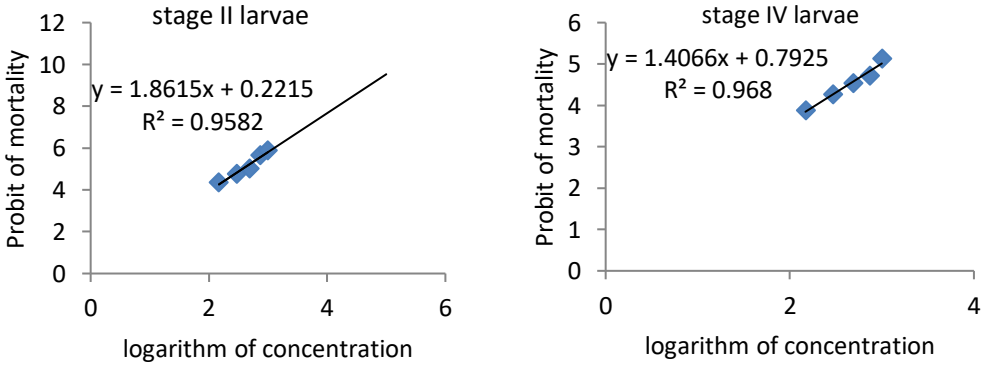


Figure 5: Regression lines for the determination of CL_{50} of essential oil from *L. martinicensis*

3.3.2. Regression lines for the determination of LH_{50} of essential oil from *L. martinicensis*

The determination of the lethal hours 50 (LH_{50}) of essential oil extracted from *Leucas martinicensis* on stage II and IV larvae of *Anopheles gambiae* ss was carried out according to the Finney method (1971) based on the regression lines

(Figure 6) obtained by transforming the percentages of mortality into probit after 24 hours of exposure based on the decimal logarithm of the hours.

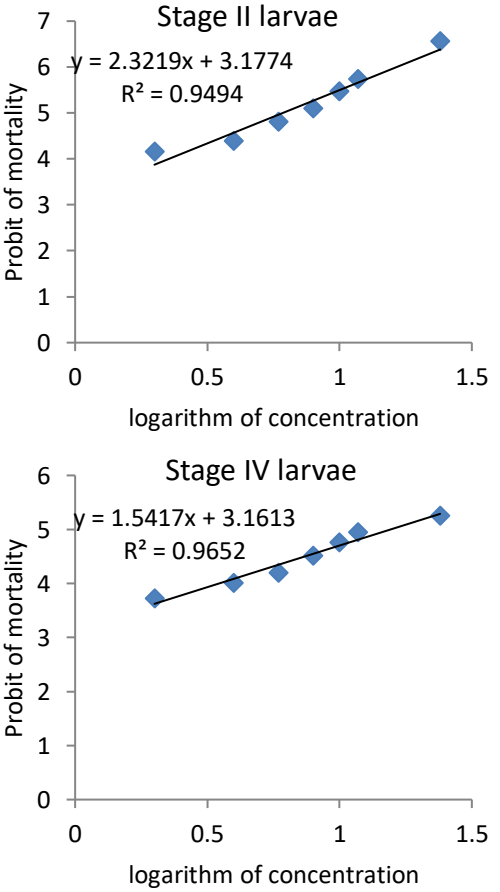


Figure 6: Regression lines for the determination of LH_{50} of essential oil from *L. martinicensis*

3.3.3. LC_{50} and LH_{50} calculated from the regression lines

Based on the regression lines determined above, the LC_{50} and LH_{50} are calculated and recorded in Table II.

Table II: LC_{50} and LH_{50} calculated and correlation between the two variables

Larval stages	LC_{50} (ppm)	LH_{50} (h/min/sec)	cor (LC_{50}, LH_{50})	t	*df* p-value	IC_{95}
II	369,81	6h 06min 05sec	0.997	23.11	3 0.00017	[0.95 – 0.99]
IV	983,75	15h 36min 33sec				

In light of the data presented in Table II, the essential oil extracted from *Leucas martinicensis* was more effective in stage II larvae with an LC_{50} of 369.81 ppm compared to stage IV where the LC_{50} (983.75 ppm) is very high. The lethal hours determined follow the same order of reactivity with 6h 06min 05sec for stage II larvae and 15h 36min 33sec for stage IV larvae. The calculation of the strength of the bond between LC_{50} and LH_{50} shows that there is a correlation between the two variables. In fact, the calculated Pearson correlation coefficient "R" was 0.997 (t= 23.11, *df* = 3, p-value = 0.0001775; CI_{95} [0.95 – 0.99]).

3.4. Phytochemical Results

3.4.1. Phytochemical Screening of *Leucas Martinicensis*

In this study, we investigated the phytochemical Screening of the essential oil extracted from *Leucas Martinicensis*. The results revealed a rich phytochemical profile in the essential oil, including polyphenols, flavonoids, tannins, saponins, and terpenoids (Table III).

Table III: Qualitative phytochemical analysis of the essential oil from *L. martinicensis*

N°	Phytochemical	*Leucas martinicensis*
1	Polyphenols	++
2	Flavonoids	++
3	Tannins	+
4	Saponins	+
5	Terpenoids	+++

(+) : presence in trace ; (++) : average presence ; (+++) : strong presence

3.4.2. Quantitative test

The results obtained (Figure 7) during the assay of chemical compounds show that terpenoids have the highest levels at 30.62±0.765g eq Lupeol/100g DM. Polyphenols come in second with a content of 17.87±0.44g equivalent of gallic acid/100g DM, followed by flavonoids (13.61±0.38g equivalent of quercetin/100g DM), tannins (8.83±0.23g equivalent of catechin/100g DM), and saponins (5.94±0.51g equivalent of galactose/100g DM).

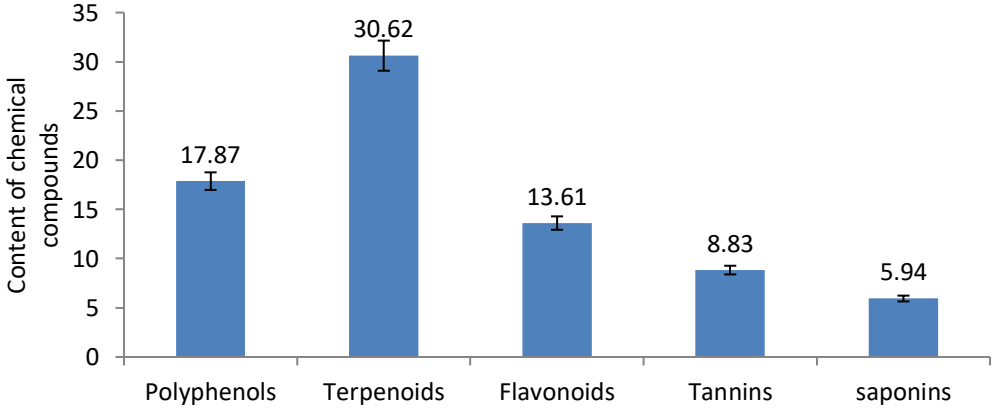


Figure 7: Content of chemical compound in the essential oil of *L. martinicensis*

4. Discussion

Significant mortalities were observed in the II and IV stage larvae of *Anopheles gambiae* ss exposed to different concentrations of essential oil extracted from *Leucas martinicensis*. Our results are similar to those of Elumalai *et al.* (2015) which show that methanolic extracts of *Leucas martinicensis* caused a mortality of 52% at a minimum concentration of 50 ppm when exposed for 24 hours. The obtained LC_{50} and LH_{50} suggest the existence of a close relationship between the two variables. Indeed, a plant essence that shows low efficacy or a high LC_{50} will also exhibit a high LH_{50} (Bouba, 2022). The results obtained are far superior to those of Njan Nlôga *et al.* (2007), who conducted a study on the efficacy of six essential oils extracted from local plants in Northern Cameroon on *Anopheles gambiae* sl and found LH_{50} ranging between 6h 36min 36sec (*Ocimum canum*) and 101h 23min 24sec (*Pittosporum viridiflorum*) with respective LC_{50} of 11.95 mg.m-2 and 71.79 $mg.m^{-2}$. The difference between the results obtained and those of Njan Nlôga *et al.* (2007) could be explained by the type of plant essence used and the species of anopheles tested. The observed mortalities can be explained by the abundance of chemical compounds in the essential oil (Bouba, 2022). This means that terpenic compounds are more active on anopheles larvae compared to phenolic compounds. Phytochemical tests have shown that *Leucas martinicensis* has a high content of terpenoids (30.62±0.765g eq Lupeol/100g DM), polyphenols (17.87±0.44g equivalent of gallic acid/100g DM), flavonoids (13.61±0.38g equivalent of quercetin/100g DM), tannins (8.83±0.23g equivalent of catechin/100g DM), and saponins (5.94±0.51g equivalent of galactose/100g DM). According to Venketachalam and Jebasan (2010), *Leucas martinicensis* contains chemical compounds that act as larvicides, repellents observed in numerous studies. In West Africa, the leaves of *Leucas martinicensis* are used to repel mosquitoes (Muhammad *et al.*, 2012). Components such as flavonoids, alkaloids, and volatile oils may be responsible for repelling adult *Culex* mosquitoes (Muhammad *et al.*, 2012). Compounds derived from this plant (triterpenes, alkaloids) act as larvicides and repellents (Gbolade, 2000 ; Venketachalam et Jebasan, 2010). Extracts from this plant have shown larvicidal activity on all larval stages of *Culex quinquefasciatus* (Battu *et al.*, 2018). It is indicated in the work of Muhammad *et al.* (2012) that the methanolic extract of *Leucas martinicensis* contains chemical constituents such as flavonoids, tannins, alkaloids, anthraquinones, volatile oils, and saponins that could be responsible for the repulsion of adult mosquitoes of the genus *Culex*.

Conclusion

The results of this study contribute to the knowledge of the chemical constituents and larvicidal activities of *Leucas martinicensis*, supporting its potential as an insecticidal plant. A deeper exploration of its phytochemical composition and larvicidal properties could lead to the development of new biocides offering advantages in the plant protection sector and its application in vector control.

Authorship

The concept was developed and planned by Professor Saotoing Pierre. The data was gathered by Souke Abraham and wrote the paper. Doctor Bouba Théophile,

Professor Saotoing Pierre and Professor Tchuenguem Fohouo Fernand-Nestor reviewed the manuscript, evaluating and interpreting the data statistically.

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Declaration of Competing Interest
No conflicting interest to declare.

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