Bioefficacy of local *Lantana camara* (Verberneae) plant extracts against the 3rd instar larva and adult stages of *Anopheles gambiae sensu lato* (Giles).

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Abstract

Resistance of malaria vectors to synthetic chemicals with high operational cost and environmental pollution has been a great challenge to scientists. Alternative approaches such as the use of natural plant products which are environmentally friendly are put in place to control malaria vectors. This study was focused on testing the effectiveness of three solvent extracts of *Lantana camara* on the 3rd instar larvae and adults of *Anopheles gambiae s. l.* These extracts were obtained by maceration. Bioassays test were carried out by WHO’s method for determination of larvicidal and adulticidal efficacy. The results show that, larval mortality increased significantly with the concentration and exposure time. Lethal concentrations 50 (LC50) and 95 (LC95) after 24 hours of larvae exposure time are respectively 0.31 g/mL and 1.53 g/mL while within 48 h they are 0.27 g/mL and 0.79 g/mL for hexane extract; 1.45 g/mL and 2.0 g/mL (24 h exposure), 0.84 g/mL and 1.55 g/mL (48 h exposure) for acetone extract; 1.96 g/mL and no lethal concentration causing 95% mortality was determined 0.40 g/mL and 2.20 g/mL (48 h) for aqueous extract. The efficacy of hexane and aqueous extract on the adult knock down and mortality were not significant even with the increasing extract concentrations and exposure time whereas with acetone extract, the adult LC50 after 24 h was 2.4 g/mL but with 95% mortality lethal concentration was not determined. According to the results, hexane extract showed high larvicidal efficacy of *An. gambiae* and acetone extract showed significant adult mortality. Those two extracts of *L. camara* can be used to fight against *An. gambiae* as alternative malaria vector control to replace conventional insecticides.

Keywords: Extracts, Lantana camara, bioefficacy, Anopheles gambiae.

1 | INTRODUCTION

Malaria remains one of the leading public health problems in Cameroon as in other parts of sub-Saharan Africa (Titanji et al., 2008) Malaria is a major public health problem with 219 million cases and 435,000 deaths in the world in 2017, which includes 90% of cases in...
Sub-Saharan Africa (WHO, 2018). Over 90% of malaria cases are estimated to occur in sub-Saharan Africa and more than 40% of the world population lives in areas prone to malaria. Children under five years old and pregnant women are the most affected (WHO, 2016). *Plasmodium falciparum* (Welch) is responsible of the majority of malaria deaths globally and is the most prevalent species in Sub-Saharan Africa (Kyalo et al., 2016). *Plasmodium vivax* is the second most significant and prevalent species in South East Asia and Latin America (Coetzee et al., 2013). *Plasmodium vivax* and *P. ovale* have the added complication of a dormant liver stage, which can be reactivated in the absence of a mosquito bite, leading to clinical symptoms. *Plasmodium ovale* and *P. malariae* represent a minimum percentage of infections. Fifth specie of malaria pathogen is *P. knowlesi*, an emerging cause of malaria in Malaysia and Southeast Asia. Previously, *P. knowlesi* infection was thought to be limited to macaques for all but the most exceptional cases; it is usually asymptomatic or results in only mild disease in these monkeys. Natural human infections have been sporadically diagnosed since 1965 (Fong, 1971), until an investigation in 2004 of atypical *P. malariae* infections led to the recognition that a large number of Malaysian patients, mostly adults, are infected with *P. knowlesi* (Singh, 2004). Retrospective studies subsequently determined that several cases of malaria previously attributed to other *Plasmodium* species were instead caused by *P. knowlesi* (Lee, 2009). *Plasmodium knowlesi* is now accounting for the majority of human malaria cases in some parts of South-East Asia. For example, it is major cause of severe and fatal malaria in Malaysian Borneo.

The distribution of vectors species of different types of malaria pathogens in the world depends of ecological and climatic factors. In the equatorial forest region, malaria is transmitted by *Anopheles gambiae*, *An. coluzzii*, *An. funestus*, *An. nili*, and *An. moucheti* (Njau Nloga et al., 1993). Although the organochlorine insecticides were widely used in agriculture and malarial control programs from the 1940 to 1960 with dramatic benefits, they fell into disfavor because of their persistence in the environment, wildlife, and humans. In the human body these insecticides induce neurotoxicity. Considerable evidence suggests that the insecticides act by altering the electrophysiological and associated enzymatic properties of nerve cell membranes, causing a change in the kinetics of Na⁺ and K⁺ ion flow through the membrane during the conduction of nerve impulses (Smith, 1991; Woolley et al., 1985). Substantial progress has been made in malaria control in the 21st century. Some studies estimate that the clinical incidence of *Plasmodium falciparum* malaria has dropped by 40% since 2000 equating to the prevention of 663 million cases (Bhatt et al., 2015). The main focus of malaria control relies on the use of long-lasting insecticidal nets (LLINs), indoor residual spraying (IRS), and treatment with artemisinin-based combination therapy (ACT). Of these methods, 68% of cases averted have been attributed to the use of LLINs (Bhatt et al., 2015). This method of malaria prevention is particularly effective in Africa where the major malaria vectors, *An. gambiae* and *An. funestus*, are largely endophagic (feed indoors) and endophilic (rest indoors after blood-feeding). Currently only one insecticide class, the pyrethroids, is commonly used to treat LLINs; pyrethroids have the required dual properties of low mammalian toxicity and rapid insecticidal activity (Zaim et al., 2000), and their repellent or contact irritant effects may enhance the personal protection of LLINs. Unfortunately, resistance to pyrethroids is now widespread in African malaria vectors (Ranson and Lissenden, 2016). The evolution of insecticide resistance and its continuing spread threatens the operational success of malaria vector control interventions. The current impact of this resistance on malaria transmission is largely not quantified and will vary depending on the level of resistance, malaria endemicity, and proportion of the human population using LLINs (Churcher et al., 2016). Since the chemical compounds used to treat mosquito nets and fight against the malaria vector

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induce negative effect in the environment and in the human healthy alternative control strategy of malaria vector can be found by using biopesticides from plant products. Natural repellents are volatile plant oils, which when used in higher concentration and frequent reaplication, effectively repel mosquitoes. Thousands of plants have already been tested as potential sources of insect repellents (Sukumar et al., 1991). For example, citronella oil, lemon oil, eucalyptus oil, cinnamon oil, castor oil, rosemary oil, lemongrass oil, cedar oil, peppermint oil, clove oil, geranium oil, and other possible oils from verbena, pennyroyal, lavender, pine, cajeput, basil, thyme, allspice, soybean, and garlic (Brown and Hebert, 1997) have been used to repel mosquitoes. The aim of this survey is to test larvicidal and adulticidal effect of Cameroonian L. camara extracts on the malaria vector An. gambiae.

2 MATERIALS AND METHODS

Preparation of plant extracts

About 10 kg of the flowers and leaves of L. camara was collected in December 2018 in Sabga locality of Tubah Subdivision, Mezam Division in the North West Region of Cameroon. The leaves and flowers were dried for two weeks under room temperature in an empty room in Bambili village. When the leaves and flowers were crispy dried, it was ground into powder with an electric blender. The extraction took place in Fundamental Laboratory of the University Institute of Technology, the University of Ngaoundere, Cameroon. Two thousand and three hundred grams of L. camara powder were mixed with 7.5 L of hexane and stirred for 30 min and allowed to stand for 24 h in the laboratory, and then re-stirred again. After 48 h, the mixture was then filtered with a filter paper Whatman n°1. The residue obtained after filtration was put through the process above again and the filtrate was admixed with the one obtained initially. After the hexane extraction was done, the paste left was dried for 10 h in the laboratory at room temperature and then used for acetone extraction and followed by aqueous extraction. The filtrates obtained with hexane, acetone and water were then separately concentrated in a rotavapor at 70°C, 60°C and 65°C, respectively at 120 rpm. Extracts were stored in a refrigerator at 4°C until used for bioassays.

Rearing of mosquitoes

Eggs of An. gambiae used for this study were obtained from the Organization and Coordination for Endemic diseases in Central Africa (OCEAC) in Yaounde. Eggs were reared in the insectary mounted in Laboratory of Zoology Higher Teachers Training College of the University of Yaoundé I. Larvae were fed with Tetramin young fish food at a rate of 2.5 mg per 100 larvae per day. The pupae were collected in plastic cups and placed in emergence cages. Adults emerging from pupae were fed using a 10% glucose solution. The colony of the laboratory strains were maintained continuously at 25-27°C and 75-78% relative humidity under photoperiod 12L:12D. Anopheles gambiae third instar larvae and female aged 2-5 days old were used to carry out bioassays. Adult females obtained from this batch were fed with blood meal to obtain eggs for the study. A rabbit was used as a source of blood meal for female reproduction. The feeding and egg maturation level was permanently maintained in the laboratory according. The eggs were laid on damp filter papers placed inside the mosquito cage. Eggs laid on the filter papers were collected the next day and conserved in Petri containers covered with paraffin within 24-36 h with an ambient temperature (25-27) °C for maturation.

Preparation of tested extract solutions

Extracts were removed from the refrigerator and allowed to attain room temperature before use. The different extracts of L. camara were dissolved in their appropriate solvents; hexane, acetone and aqueous solvents. A series of 200 mg, 400 mg, and 600 mg of each extract were measured using an electronic scale balance into small screw-cap bottle vials to avoid evaporation of volatile solvents. Each measured extract was then serially dissolved in 20 ml of absolute alcohol and poured into dry test tubes to avoid evaporation of volatile solvents. The solutions were shock vigorously to dissolve or disperse the material in the solvent in order to obtain a homogenous solution. One hundred and fifty milliliters disposable plastic cups were placed on the working table, each
labeled with the concentration (between 0.5 g/mL to 3.5 g/mL) of the particular stock solution of the different extracts (hexane, acetone and aqueous). Three replicates of the test cups were then set up for each concentration and an equal number of controls set up simultaneously (the control set up is per extract and not concentrations as its result will apply to all concentrations of a particular extract) and the cups arranged in an orderly manner to avoid errors. Ninety nine (99) mL of spring water was measured using a measuring cylinder and put in each cup and the depth of the water in the cups maintained between 5 cm and 10 cm as deeper levels may cause undue mortality of the larvae. In each of the test cups, 1 mL of the different concentrations (0.5 g/mL, 1 g/mL, 2 g/mL, 2.5 g/mL, 3 g/mL and 3.5 g/mL) of the appropriate stock solutions was added using a micropipette into their corresponding cups as labeled to form the test solutions. In each of the control cups of the different extracts, 1 mL of ethanol was added to the 99 mL of spring water to make up the control solution.

Bioassays

The third instar larvae were used to assess the larvicidal activity of *L. camara* plant extracts following WHO guidelines for laboratory and field testing of mosquito larvicides (WHO, 2005). An initial survey was carried out with a wide range of test concentrations and a control to find out the activity range of the materials under test. After determining the mortality of larvae in this wide range of concentrations, a narrower range of 5 – 6 concentrations were used to determine lethal concentrations causing 50% and 95% larval mortality (LC$_{50}$ and LC$_{95}$) during 24 h or 48 h respectively. Twenty five 3rd instar and healthy larvae were picked up from the basins at random using a dropper and first placed in a petri dish. The larvae were then transferred into all the cups at about the same time and the time of contact with the solutions noted. Observation for mortality was done after 24 h and 48 h and the number of dead and life larvae were counted and recorded using a long needle. Moribund larvae were also counted and recorded as dead when calculating percentage mortality. The following formula was use to calculate mortality percentages.

\[
\text{% mortality} = \frac{\text{number of dead larvae}}{\text{total number of larvae}} \times 100
\]

Bioassays were held at 25–28°C and a photoperiod of 12 h light followed by 12 h dark. After 48 h, the cups and their contents were discarded properly. Adult bioassays were performed with 2-5 days-old non-blood-fed females following the WHO Guidelines (WHO, 2013). The stock solutions as prepared above were obtained in their different test concentration: 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5 g/mL and allowed to stand on a test tube rag. Non impregnated nets were measured in circular small sizes and completely immersed into each test tube containing appropriate concentrations of the stock solutions. The solutions were well shacked with the nets inside and covered with cotton to avoid evaporation of volatile solvent and then allowed to stand for 1h 30 min on the test tube rag. The control solution was also prepared with absolute ethanol and the net immersed in, covered and allowed to stand for same time as the test solutions. The nets were later removed from the test and control solutions with a forcep and spread on dry empty petri dishes to air dry for 24 h at room temperature. Series of 25 non blood fed adult females aged 2-5 days old were aspirated from the cage with a mouth aspirator. It was then transferred into an already prepared disposable cups (labeled cups covered with non-impregnated nets and an opening at the side covered with a piece of paper) and allowed to stand for 1h in other to observe for unhealthy adults to be replaced with healthy ones. The dried impregnated nets were placed on an inclined wooden board at 45° protected by a plastic card and then covered with plastic cones. It was held firmly on top of the board with a paper scorch to avoid the cones from falling off and escaping of mosquitoes. The small openings of the cones were covered with cotton wool. The 25 mosquitoes were then transferred into each cone with the aspirator by blowing the mosquitoes gently and the time of contact with the treated nets noted. The labels on the test tubes were also transferred to their respective cones to avoid errors. An observation for knocked down mosquitoes was carried out at 10 min intervals during a period of 1 h of contact with the impregnated nets and the number of knockdown mosquitoes was recorded. At the end of the 60 min, the mosquitoes were aspirated from the cones and transferred back into their corresponding cups. They were provided with 10% sucrose sucked in small cotton wools and placed on top of the non-impregnated net over the
cups. The cups were then allowed to stand for 24 h to check for mortality (dead and moribund adults). During this period, the holding tubes were kept in shady and sheltered place in the laboratory maintained at 27°C ± 2°C temperature and 75% ± 10% relative humidity. The temperature and humidity was recorded during the recovery period. At the end of the recovery period (24 h post-exposure), the number of dead mosquitoes were counted and recorded. An adult mosquito was considered to be alive if it is able to fly regardless of the number of legs remaining. Any knocked down mosquito, whether or not they have lost legs or wings, are considered moribund and was counted as dead. **Statistical analysis**

The data was analyzed using SPSS software version 17.0. Chi-square test, used to calculate the frequency of tested variables while the Wilcoxon Z test was used to compare average mortality rates between individuals. Dose-mortality regressions were computed by probit analysis using WinLD software (CIRAD, Montpellier, France). Results were expressed as percentage mortalities, corrected for untreated mortalities using Abbott’s formula. LC50 (Lethal Concentration to kill 50% of insects) and LC95 (lethal concentration to kill 95% of insect) were recorded to evaluate the exact efficacy of the solvents extracts toxicity. P- Value was set at 5%.

### RESULTS

#### Table 1: Larvae mortality as a function of exposure time to different concentrations of aqueous extract

| Extract/concentration (g/mL) | 24hrs | 48hrs | Test | % Mortality (mean ±SE) |
|-----------------------------|-------|-------|------|------------------------|
| 0                           | 0.00±0.00 | 0.00±0.00 | - | - |
| 0.5                         | 4.00±1.63 | 61.50±1.4A | 3.70*** |  |
| 1                           | 21.00±1.08 | 68.75±1.4A | 1.85** |  |
| 1.5                         | 8.00±0.32 | 74.75±1.5A | 3.50** |  |
| 2                           | 1.00±2.30 | 86.25±3.51A | 1.19** |  |
| 2.5                         | 11.00±1.73 | 81.25±3.50A | 1.95*** |  |
| 3                           | 7.00±0.42 | 48.75±3.25A | 8.86*** |  |

**FIGURE 1:** Larvicidal effect of L. camara aqueous extract on An.gambiae s. l. after 24 and 48 h.

According to the Figure 1 above, the difference between mortality rate (assessed with 95% confidence interval) is significant even for 24 h exposure (H=4.14; p=0.014) or for 48 h exposure (H=7.04; p=0.002). The regressions line equations generated are: \( Y_{aq}=0.2+10X \) and \( Y_{aq}=24.9+27X \) respectively for 24h and 48 h exposure period. The lethal concentration causing 50% mortality of the exposed larvae after 24 h is 1.96 g/mL and 0.40 g/mL in 48 h exposure. The lethal concentration causing 95% mortality of the exposed larvae after 24 h is not determined but with 48 h exposure it is 2.20 g/mL.

#### 3.1. Larvicidal activity of the different Lantana camara extracts on Anopheles gambiae senso lata

- **Larvicidal effect of aqueous extract**

According to the results obtained (Table 1), within 24 h exposure, there is increasing of larvae mortality with increasing of extract concentration but after 2.0 g/mL of concentration the larval mortality decreases with the increasing of extract concentration. The same situation was observed with 48 h exposure to the aqueous extract. This can be explained by the fact that may be the homogeneity of aqueous solvent with the extract is not effective.

- **Larvicidal effect of acetone extract**
According to the results of table 2 below, the larvicidal effect of acetone extract on the 3\textsuperscript{rd} instar larvae during 24 h and 48 h periods show significant increase in percentage mortality with augmentation in extract concentration and time exposure.

**TABLE 2:** Larvae mortality as a function of exposure time to different concentrations of acetone extract

| Extract concentration (g/mL) | % Mortality (mean±SE) | 24h | 48h | T test |
|-----------------------------|-----------------------|-----|-----|-------|
| 0                           | 0.00±0.00D            | 0.00±0.00D | -    |
| 0.8                         | 11.00±1.12D           | 37.52±3.77C | -2.50*|
| 1                           | 31.00±3.42CD          | 59.13±2.96C | -6.22***|
| 1.4                         | 48.00±4.64BC          | 90.57±4.21B | -4.43**|
| 1.6                         | 69.00±3.29B           | 97.91±1.21AB | -3.09*|
| 2                           | 95.00±2.52A           | 100.00±0.00A | -1.99ns|
| 3                           | 100.00±0.00A          | 100.00±0.00A | -    |
| F(7, 24)                    | 75.83***              | 84.38*** | -    |

Means ±SE in the same column followed by the same upper case letter do not differ significantly (Tukey’s test; p<0.05). ns P> 0.05; * p< 0.05; **p<0.001; *** p<0.001.

From the figure 2, the difference between mortality rate is significant for 24 h exposure (H=3.59; p=0.015) and for 48 h exposure period (H=6.97; p=0.0009). The regression line equations corresponding to exposure duration are: \( Y_{ac1} = -49.85+72.14X \) and \( Y_{ac2} = -0.35+55.14X \) respectively for 24 h and 48 h exposure. The lethal concentrations necessary for 50% and 95% mortality after 24 h exposure are: 1.45 g/mL and 2.0 g/mL respectively. For 48 h exposure, the lethal concentrations for 50% and 95% mortality are: 0.84 g/mL and 1.55 g/mL respectively.

**- Larvicidal effect of hexane extract**

With hexane extract on the 3\textsuperscript{rd} instar larva as shown on table 3 below, within 24 h and 48 h period of exposure, there is significant increasing of larval percentage mortality with increasing of extract concentration and with increasing of exposure duration. However, high percentage mortality was recorded with lower concentrations within 24 h and 48 h.

**TABLE 3:** Larvae mortality as a function of exposure time to different concentrations of hexane extract

| Extract concentration (g/mL) | % Mortality (mean±SE) | 24h | 48h | T test |
|-----------------------------|-----------------------|-----|-----|-------|
| 0                           | 0.00±0.00D            | 0.00±0.00D | -    |
| 0.4                         | 62.54±5.30C           | 74.01±8.54C | -1.14ns|
| 0.6                         | 68.24±9.96BC          | 84.82±6.72BC | -1.38ns|
| 0.8                         | 90.50±4.51A           | 95.65±3.07AB | -0.94ns|
| 1.6                         | 98.91±1.09A           | 100.00±0.00A | -1.00ns|
| 1.8                         | 100.00±0.00A          | 100.00±0.00A | -    |
| 2                           | 100.00±0.00A          | 100.00±0.00A | -    |
| 3                           | 100.00±0.00A          | 100.00±0.00A | -    |
| F(8, 27)                    | 58.84***              | 69.58*** | -    |

Means ±SE in the same column followed by the same upper case letter do not differ significantly (Tukey’s test; p<0.05). ns P> 0.05; *** p<0.001.

**FIGURE 2:** Larvicidal effect of L. camara acetone extract on An.gambiae s. l. after 24 and 48 h.
BIOEFFECTIVITY OF LOCAL LANTANA CAMARA (VERBERNEAE) PLANT EXTRACTS AGAINST THE 3RD INSTAR LARVA AND ADULT STAGES OF ANOPHELES GAMBIAE SENSO LATO (GILES).

FIGURE 3: Larvicidal effect of L. camara hexane extract on An.gambiae s. l. after 24 and 48 h.

The difference of mortality rate between concentrations is significant for 24 h exposure time (H=10.5; p=0.0001) and 48 h exposure (H=23.39; p<0.001). The regression lines equations are: $Y_{hex1}=41.51+34.82$ and $Y_{hex2}=73.50+15.96$ respectively for 24 h and 48 h exposure time. The lethal concentration for 50% and 95% mortality after 24 h exposure are: 0.31 g/mL and 1.53 g/mL respectively. For 48 h exposure the lethal doses to cause 50% and 95% larval mortality are: 0.27 g/mL and 0.79 g/mL respectively.

3.2. Adulticidal effect of Lantana camara plant extracts

Adult Knock down by Lantana camara extracts

The activities of the three solvent extracts tested on the adults and knock down recorded at 10 min intervals is shown on the Table 4. The results of the knock down when compared with the control showed that, all solvent extracts generally caused no significant adult knock down. The results obtained is independent on the concentration and post exposure time as even higher concentration of 3.5 g/mL hexane caused 0.00% knockdown at time 10 min and 3.00% after 60 min in which there is no significance (ns). For 3.5 g/mL of both acetone and aqueous extract, the percentage of knock downs for 10 min and 60 min are 0.00% and 5.00%, 0.00% and 4.00% respectively which is also no significant.

| TABLE 4: Percentage adult knockdown as a function of exposure time to different concentrations of acetone extract |
|---|---|---|---|---|---|
| Extract conc (g/mL) | 10 min | 20 min | 30 min | 40 min | 50 min |
| 0 | 0.00|0.00 | 0.00|0.00 | 0.00|0.00 | 0.00|0.00 | 0.00|0.00 |
| 1 | 0.00|0.00 | 0.00|0.00 | 0.00|0.00 | 0.00|0.00 | 0.00|0.00 |
| 2.5 | 0.00|0.00 | 0.00|0.00 | 0.00|0.00 | 0.00|0.00 | 0.00|0.00 |
| 3 | 1.00|1.00 | 2.00|1.15 | 3.00|1.00 | 4.00|1.15 | 5.00|1.15 |
| 3.5 | 0.00|0.00 | 1.00|1.00 | 2.00|1.15 | 3.00|1.31 | 4.00|2.31 |
| $F(4, 15)$ | 1.00*1 | 1.05*1 | 1.36*1 | 1.05*1 | 1.00*1 |

| TABLE 5: Percentage adult knockdown as a function of exposure time to different concentrations of aqueous extract |
|---|---|---|---|---|---|
| Extract conc (g/mL) | 10 min | 20 min | 30 min | 40 min | 50 min |
| 0 | 0.00|0.00 | 0.00|0.00 | 0.00|0.00 | 0.00|0.00 | 0.00|0.00 |
| 1 | 0.00|0.00 | 0.00|0.00 | 0.00|0.00 | 0.00|0.00 | 0.00|0.00 |
| 2.5 | 0.00|0.00 | 1.00|1.00 | 2.00|1.15 | 3.00|1.31 | 4.00|2.31 |
| 3 | 1.00|1.00 | 2.00|1.15 | 3.00|1.31 | 4.00|1.31 | 5.00|1.91 |
| 3.5 | 0.00|0.00 | 1.00|1.00 | 2.00|1.15 | 3.00|1.31 | 4.00|2.31 |
| $F(4, 15)$ | - | 0.89*1 | 0.30*1 | 0.14*1 | 0.22*1 |

- Adult mortality of Lantana camara extracts

From the results obtained in Table 7, the adult mortality during 24 h period exposure shows slight significant difference with respect to the solvent extracts and the concentrations. Acetone extract recorded a mortality of 67.69% against the An. gambiae adult stage with increased concentration of 3.5 g/mL and 14.43% with 1.0 g/mL of the extract.
No significant mortality was recorded for hexane and aqueous extract; however, hexane yielded higher percentage mortality of 25.64% and 34.39% for 1 g/mL and 3.5 g/mL respectively compared to aqueous extract with 5.44% and 13.29% for 1 g/mL and 3.5 g/mL respectively. The lethal concentration of \textit{L. Camara} acetone extract for 50% mortality after 24 h exposure is 2.4 g/mL while with 95% mortality lethal concentration of acetone extract was not determined.

**TABLE 7:** Percentage adult mortality as a function of exposure time to Lantana camara plant extracts

| Extract Concentration (g/mL) | Adult Mortality/24h |
|------------------------------|---------------------|
|                              | Acetone             | Aqueous             | Hexane             |
| 0                            | 0.00±0.00           | 0.00±0.00          | 0.00±0.00         |
| 1                            | 14.43±4.23         | 5.44±3.26         | 25.64±2.44       |
| 2.5                          | 50.20±7.04         | 7.76±1.07         | 35.47±3.33       |
| 3                            | 57.81±4.18         | 9.00±3.22         | 37.65±4.24       |
| 3.5                          | 67.69±3.11         | 13.29±3.03        | 34.39±4.09       |
| (4, 15)                      | 37.04**            | 3.81***           | 23.46*           |

*Means ±SE in the same column followed by the same upper case letter do not differ significantly (Tukey’s test; p<0.05). **p<0.001; *** p<0.001.

4 | DISCUSSION

The efficacy of plant extract is noticed when the lethal concentration causing 50% and 95% mortality of the targeted organism during 24 h and 48 h is low. According to the results obtained in this study, the hexane extract is more effective to fight against larvae of \textit{An. gambiae} than the aqueous and acetone extracts of \textit{L. camara} and the acetone extract been more effective than aqueous extract. Lantana camara aqueous extract showed limited efficacy in the fight against the larvae of \textit{An. gambiae} because no concentration induces 95% of larval mortality. This means during the extraction with water as solvent many active components remain incorporate in the plant tissues and this bring the plant extract to not be effective to fight against \textit{An. gambiae} larvae. The appropriate solvent recommended to prepare \textit{L. camara} extract to control \textit{An. gambiae} larvae is hexane. With \textit{L. camara} hexane extract the obtained lethal concentrations causing 50% and 95%larval mortality are: 0.31g/mL and 1.53g/mL in 24h; 0.27g/mL and 0.79g/mL in 48 h. Among the three \textit{L. camara} extracts not none had given lethal concentration causing 50% and 95% of adult mortality except acetone extract which induced 50% of adult mortality with a concentration of 2.25g/mL. Thus to obtain high number of adult mortality, extract concentration and exposure time should be increased. Comparing our results with the results obtained by Abe et al. (2018) on the insecticidal activity of \textit{Cannabis sativa} L. leaf essential oil on the malaria vector \textit{Anopheles gambiae}, it is noticed that the lethal concentration causing 50% of larval mortality is lower for \textit{L. camara} hexane extract than lethal concentration of \textit{C. sativa} essential oil in 24h while lethal concentration causing 95% of larval mortality is lower for \textit{C. sativa} essential oil than lethal concentration of \textit{L. camara} hexane extract within 48 h. It can be suggested that the larvicidal activity of the two biopesticide is similar but faster with \textit{L. camara} hexane extract and \textit{C. sativa} essential oil. But concerning adulticidal activity, \textit{C. sativa} essential oil is more effective than \textit{L. camara} hexane extract. Bioassays conducted with adults indicated knock down times for 50% and 95% of 23 min and 28 min respectively and lethal concentrations killing 50% and 95% of adult mosquitoes were low with \textit{C. sativa} while with \textit{L. camara} hexane extract no specific time was determined to induce 50% and 95% adults knock down also no lethal concentration killing 50% and 95% of adult mosquitoes were determined. According to Abe et al. (2019), \textit{Piper umbellatum} leaf essential oil was effective against the third instar larvae and adult females of \textit{Anopheles gambiae} S.L and \textit{Culex quinquefasciatus} while \textit{L. camara} acetone and hexane extracts were effective against the third larvae but not effective with adults of \textit{Anopheles gambiae}. Foko Dadji et al.(2011) also reported that, \textit{Capsicum annuum}, \textit{Piper nigrum}, and \textit{Zingiber officinale} essential oils are very useful to control adults of \textit{An. gambiae} comparatively with \textit{L. camara} extracts which are not effective with adulticidal activity on \textit{An. gambiae}. The insecticidal activity of essential oils depends of the plant species used to fight against vector of malaria and also...
depends of vector species. According to Foko Dadji et al. (2018), Clausena anisata essential oil is fully effective with low concentration (100% mortality) when it is tested against third instar larvae of An. coluzzii in 48 h exposure while L. camara hexane extract induces 100% An. gambiae larval mortality with high concentration in 48 h exposure.

5 | CONCLUSION

This survey examined the effectiveness of L. camara extracts with different solvents on the larvae and adults of An. gambiae rearing in laboratory. The results show that among the three extracts, L. camara hexane extract induces 50% and 95% larval mortality in 24 h and 48 h exposure with low concentration comparatively with acetone and aqueous extracts. Only acetone extract induces 50% of adult mortality at 2.4 g/mL in 24 h exposure, no extract concentration induces 95% adult mortality in 24 h exposure. Then the adulticidal activity of L. camara extracts is not effective to fight against An. gambiae adults. To prepare a biopesticide for the control of An. gambiae larvae, the hexane extract of L. camara is recommended.

6 | ACKNOWLEDGEMENT

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