Biological activities of the essential oils of *Cupressus macrocarpa*, *Lantana camara* and *Psidium littorale* against *Plasmodium falciparum* welch, 1897 and *Anopheles gambiae* giles, 1902

Gaëlle Magne Tamdem, Patrick Akono Ntonga, Henri Gabriel Tsila, Calvin Tonga, Pasma Mache Nkouandou, Christelle Awansi Djeukam, Rachel Ngaha, Odette Etoile Ngo Hondt, Romeo Mbongue, Willy Teukam Soh, Ronny Kojom Kamga, Loïc Kojom Foko, Pierre Michel Jazet Dongmo and Chantal Menut

DOI: [https://doi.org/10.22271/j.ento.2020.v8.i6l.7949](https://doi.org/10.22271/j.ento.2020.v8.i6l.7949)

Abstract

This study assessed the *in vitro* anti-plasmodial on and larvicidal activity of essential oils from the leaves of *Cupressus macrocarpa*, *Lantana camara* and *Psidium littorale*. The chemical compositions of the different oils were determined. The toxicity of the oils was tested on *An. gambiae* larvae according to WHO protocol and their *in vitro* anti-plasmodial activity was assessed by radio-isotopic method. α-pinene (20.78%), β-caryophyllene (20.37%) and 1,8-cineole (eucalyptol) (39.55%) were the major compounds in *Cupressus macrocarpa*, *Lantana camara* and *Psidium littorale* oils respectively. The *in vitro* anti-plasmodial activity showed that *Lantana camara* essential oil is more effective (IC50=12.34 ppm) than *Cupressus macrocarpa* (147.29 ppm) and *Psidium littorale* (115.45 ppm). Essential oil of *Lantana camara* showed higher activity on larvae from Yassa (DL=7.37 ppm) while that of *Psidium littorale* was more active on larvae from Youpwe (DL=49.2 ppm). These oils can be used for the development of new biocides and natural anti-malarial drugs.

Keywords: *Cupressus macrocarpa*, *Lantana camara*, *Psidium littorale*, *Anopheles gambiae* s.l., larvicidal activity, anti-plasmodial activity

Introduction

More than a century after the discovery of its causal agent and the role of the mosquitoes belonging to the ‘*Anopheles*’ genus in its transmission, malaria remains one of the most dreaded diseases [1]. Approximately 154-289 million people are infected each year, with 80% of cases occurring in sub-Saharan Africa. Children less than 5 years old, pregnant women, people living with HIV/AIDS, naive migrants, mobile populations and travellers are the most vulnerable [2]. Endemic countries are deploying various malaria control means including vector control activities and adequate patient management based on early diagnosis and administration of effective therapies. Despite the 21% drop in prevalence recorded worldwide between 2010 and 2015 [3], the situation remains a cause for concern. This may owe to rough application of the recommended preventive and curative measures, and above all, in the dual resistance of vectors to insecticides and *Plasmodium* to antimalarial drugs. With regard to vectors, use of DDT and pyrethroids in both agriculture and public health has resulted in the selection of resistant strains [4, 5]. In recent years, the emergence of *Anopheles* strains resistant to synthetic insecticides has been reported in many African countries including Benin [6], Côte d’Ivoire [7], Niger [8], Nigeria [9], Equatorial Guinea [10] and Cameroon [11, 12]. With regard to the parasite, it is known that self-medication, utilization of drugs from street vendors and non-compliance with prescribed doses are responsible for the development of resistant strains of *Plasmodium* [13]. The emergence and spread of resistant strains of *Plasmodium* and vectors in sub-Saharan Africa is jeopardising malaria control efforts in endemic countries. In this context, the search for natural molecules with effective biological properties is essential. Plants from the Cameroononian flora have for millennia been an inexhaustible source of new molecules that simply need to be explored. Numerous studies have been carried out, highlighting the insecticidal activity of plant species from Cameroononian flora [14, 15, 16, 17].
They demonstrated that plants insecticidal activities boosted when used in the form of essential oils [19]. Volatile essences have several functional groupings and can easily diffuse across the cell membranes [19]. Essential oils can therefore be useful for malaria treatment and vector control [20, 15].

**Cupressus macrocarpa**, *Lantana camara* and *Psidium littorale* are plants of the Cameroonian flora traditionally used by the population as insect repellents and treatment of many diseases including amoebiasis and malaria [21]. The traditional use of these plants attest to their potential as reservoirs of active molecules against malaria parasites and vectors. However, this is still to be investigated. The present study aims to evaluate the *in vitro* insecticidal and antiplasmodial activities of essential oils from *Cupressus macrocarpa*, *Lantana camara* and *Psidium littorale* leaves.

**Materials and Methods**

**Collection of plants and extraction of essential oils**

The plants were chosen because of their traditional use as insect repellents and treatment of certain diseases in many villages of the West region of Cameroon. Plants were collected in September 2018 in Bandjoun and Douala, free of insecticide treatment. The specimens were then identified by botanists from the Department of Plant Organism Biology of the University of Douala. The leaves of each plant species were washed with spring water, cut into small units and then subjected to hydrodistillation for 5 hours using a Clevenger-type equipment. The essential oil collected at the end of the distillation process was filtered on an anhydrous sodium sulphate column, then stored in a dark, hermetically sealed glass bottles at 4°C.

**Analysis of the chemical composition by GC and GC/MS**

The chemical analysis of essential oils was carried out using a Varian CP-3380 type chromatograph equipped with a flame ionisation detector and a capillary column (length 30 m, internal diameter 0.25 mm) with an apolar stationary phase of the methylsilicone type (DB-1, film thickness 0.25 μ). Nitrogen was used as carrier gas with 0.8 ml.min⁻¹ flow rate. The temperature of the injector was 220°C; the detector at 250°C. The furnace was programmed from 50°C to 200°C with a temperature gradient of 5°C.min⁻¹. The retention indices of the different constituents was calculated in relation with the retention times of a series of n-alkanes and their relative percentages calculated by electronic integration, considering that their response factors are all equal to 1. The gas chromatography-mass spectrometry coupling was done using a Hewlett Packard HP 5970 A apparatus, equipped with an apolar capillary column (30 m x 0.25 mm) in HP-1 fused silica (film thickness 0.25 μ) and a quadrupole detector (ionisation energy 70 eV). The temperature of the injector was 220°C and that of the interface area was 210°C. Injection in split mode (1/100) of 1 μl of a 10% solution of essential oil in dichloromethane. The furnace temperature was programmed from 70°C to 200°C with a gradient of 10°C.min⁻¹. Helium was used as carrier gas with 0.6 ml.min⁻¹ flow rate. The acquisition was performed in scan mode [35-300 amu] at 2.96 scan/sec⁻¹.

The identification of the constituents of the essential oils was made on the basis of their retention indices and mass spectra by comparison with the data from literature [22].

**Collection sites for larvae of *Anopheles gambiae* s.l.**

Larval collection took place in 2 districts of Douala, namely Yassa and Youpwe. Yassa (3°58 N, 9°49 E) is a peri-urban district in the east of the city. Main activities are agriculture, animal husbandry and trade. This district is also characterised by the presence of soap and oil companies. Pot holes and old vehicle tyres, are main breeding sites for mosquitoes, especially in the rainy season. Youpwe (04°00’N, 09°42’E) is a densely populated district located in the Wouri river estuary. Urbanisation is poorly controlled and the population lives mainly from fishing and petty trade. The Wouri River is the only permanent breeding ground for mosquito larvae in this area, although some temporary breeding sites may be visible during the rainy season.

**Collection and rearing of Anopheles larvae**

Populations of *Anopheles* species for testing were collected in the larval stage in natural deposits (sewers, gutters, drums, pits, old tyres, pits and tracks, pot holes) using the dipping method [23]. Collection took place during the short rainy season (May to July 2019) at the rate of five consecutive days per month, simultaneously in the Youpwe and Yassa. Anopheles larvae were reared in water from the lodges and fed on Tetrababy fish food [24]. The adults obtained were morphologically identified [25, 26]. The males and females of *An. gambiae* s.l. were crossed to obtain F1 generation larvae that were later on tested.

**Cultivation of Plasmodium falciparum**

The chloroquine-resistant FcB1/Colombia strain of *P. falciparum* was maintained on human red blood cells in RPMI 1640 medium, containing 25 mM HEPES, pH 7.3, 2 g/L sodium bicarbonate, 2 g/L glucose, penicillin and streptomycin [27]. The medium was enriched with 10% heat-activated human serum. The RBCs and serum used came from the *Etablissement Français du Sang*. The culture was conducted at 2% haematocrit, in 25 and 75 mL vials and maintained in an oxygen-deficient atmosphere at 37°C. The culture medium was renewed once a day. A Giemsa stained blood smear was taken daily to control parasitaemia.

**Larvicidal tests**

These tests consisted of evaluating the mortality of mature stage larvae (L3 and L4) of *Anopheles* in the presence of diluted solutions of essential oils according to WHO protocol [28]. Twenty larvae were sampled using a Pasteur pipette and placed in bowls of 8 cm diameter each containing 99 ml of well water to which 1 ml of diluted test solution was added. Preliminary experiments enabled the selection of a range of concentrations for the tests. Stock solutions of essential oils from each sample were prepared in 90° ethanol. From these, dilutions were made to obtain final experimental concentrations of 50, 100 and 150 ppm. Three repetitions were carried out for each dilution. Two control bowls were also prepared under the same conditions as the test bowls. These negative control contained only ethanol (in the same proportions as for the tests, i.e. 1%) with no trace of essential oil. Larval counts were carried out every 5 minutes for 1 hour; then every hour for 10 hours and finally after 24 hours of exposure to volatile extracts solubilised in water.

**Antiplasmodial test**

The *in vitro* anti-plasmodial activity of essential oils has been evaluated by the radioisotopic method [29]. This method determines the inhibition of parasite growth in culture in the presence of various concentrations of molecules by measuring...
the incorporation of [3H] hypoxanthine in the parasite nucleic acids. The experiment was carried-out in 96-well plates as described by Guillon et al. [36]. Briefly, serial dilutions of essential oils were prepared in culture medium and added to asymptomatic parasite cultures (1% parasitaemia, 1% final haematocrit, 200 μL final volume per well) for 24 h, at 37°C, before addition of 0.5 μCi of [3H] hypoxanthine (1.5 Ci/mmol, Amersham, Les Ulis, France) per well for 24 h. The plates were incubated at 37°C in a humid, oxygen-deficient atmosphere. Freezing plates at -80°C interrupted the experiments. After thawing, the contents of the wells were collected on glass fibre filters (Wallac®, USA) using a cell collector (Filter Harvester, USA). After adding scintillation fluid (Perkin-Elmer®, USA), the radioactivity (counts per minute) was measured using a spectrophotometer (450- Microbeta Trilux, USA). The growth inhibition for each concentration was determined by comparing the radioactivity incorporated in the treated culture with that of the control (essential oil-free) culture on the same plate. The concentration causing 50% inhibition (IC50) was obtained from the drug concentration-response curve and the results were expressed as the mean ± standard deviations determined from at least three independent experiments.

To avoid inhibition of parasite growth due to diffusion of essential oils from neighbouring wells, preliminary tests were conducted to determine the highest essential oil concentration for which such inhibition was not measured. For this purpose, the crude essential oil was only diluted in series with culture medium in one row of a plate, the other row containing only culture medium. Parasites were added to all wells of the plate and the plate was treated as described above. The highest concentration of essential oil showing no inhibition of parasite growth in the surrounding wells was used as a starting concentration to further determine the intrinsic anti-plasmodial activity of the essential oil.

Statistical analysis
Statistical analyses were carried out using Statview version 5.0 software (SAS Institute, Inc, USA). Kruskal Wallis H-tests was used to compare larval mortalities. The regression curve from Henry's simplified table which transforms the mortality percentages into probits [31] made it possible to determine the LC50 and LC95. Statistical significance was set at a probability value of less than 0.05.

Results and Discussion
The present study shows that the leaves of Cupressus macrocarpa (0.673%) have a higher essential oil content than those of Psidium littorale (0.118%) and Lantana camara (0.076%) (Table 1). These yields contrast with results from Sousa et al. [32], Lídia et al. [33], and Nacira & Yousra [34]. The difference in yields can be related to extraction method, climatic conditions, geographical location of the harvest site, harvest period, and physiopathological state of the plant at the time of harvest [35, 36].

Table 1: Data on essential oils from plants collected in Cameroon

| Plant            | Collection       | Essential oil |
|------------------|------------------|---------------|
| Family           | species          | Organ         | Area  | Date      | Color | Outputs |
| Verbenaceae      | Lantana camara   | Leaves        | Douala| 24.09.2018| yellow| 0.076 % |
| Cupressaceae     | Cupressus macrocarpa | Leaves     | Bandjoun| 18.09.2018| yellow| 0.673 % |
| Myrtaceae        | Psidium littorale | Leaves        | Douala| 26.09.2018| yellow| 0.118 % |

Chemical composition
The chemical composition of the essential oils of the three plants is given in Table 2. Monoterpenes constitute the major fraction (50.27% - 89%). These monoterpenes were dominated by α-pinene (20.78%) in the oil of Cupressus macrocarpa. Similar results were obtained with samples from Argentina and Greece [36, 37, 38]. In addition, it should be noted that α-pinene has been reported to be the major component of essential oils of other species of the genus Cupressus, notably Cupressus arizonica from Tunisia, Cupressus atlantica from Morocco and Cupressus simpervirens from Algeria [39, 40]. From the above, it should be suggested that α-pinene is the characteristic molecule of the essential oils of Cupressus, although it was shown that neral and α-terpineol were the major compounds in Cupressus macrocarpa from Egypt [41, 42]. β-Caryophyllene (20.37%) was the majority compound in the essential oil of Lantana camara. Some works have shown a certain variability in the chemical composition of this plant species according to the collection site with β-caryophyllene as the majority compound for the sample from Cuba [44], (E)-nerolidol for the sample from Nepal, sabinene for the sample from Yeme [45].

Psidium littorale is mainly composed of 1,8-Cineole (eucalyptol) (39.55). Although this result corroborates those recorded by Scur et al. [46] and Marques et al. [47], it contrasts with the results recorded by Soliman et al. [48] and Adam et al. [49]. The later showed that β-Caryophyllene was the major compound in the samples of Psidium littorale originating from Egypt and French Polynesia. Thus, there is a certain variability in the chemical composition of Psidium littorale oil according to the collection site.

Table 2: Chemical composition of essential oils of Cupressus macrocarpa, Lantana camara and Psidium littorale.

| Compounds             | % Composition | Cupressus macrocarpa | Lantana camara | Psidium littorale |
|-----------------------|---------------|----------------------|----------------|------------------|
| **Monoterpenes**      |               |                      |                |                  |
| 911 Tricycyle         | --            | --                   | --             | --               |
| 928 α-thujene         | 1.51          | 0.52                 | --             | 4.36             |
| 936 α-pinene          | 20.78         | 3.9                  | --             | --               |
| 949 α-lenchene        | 0.45          | --                   | --             | --               |
| 951 camphene          | --            | 1.46                 | --             | --               |
| 963 Furfural -S-methyl| --            | --                   | 1.37           | --               |
| 978 β-pinene          | 8.81          | 17.99                | 0.32           |                  |
| 990 myrcene           | 3.64          | 1.93                 | 0.41           |                  |
| 1,006 p-Terpineïne    | --            | 0.18                 | --             |                  |
| 1,007 α-phellandrene  | --            | --                   | 0.81           |                  |
| Compound                        | δ-3-carene | 1.5 |            |
|--------------------------------|------------|-----|------------|
| 1,019 α-terpinene              | 1.8        | 0.28|            |
| 1,026 P-cymene                 | 0.95       | --  |            |
| 1,033 Limonene                 | 9.96       | --  |            |

**Oxygenated monoterpenes**

| Compound                        | 1,8-Cineole (eucalyptol) | --  | 39.55 |
|--------------------------------|--------------------------|-----|-------|
| 1,046 cis-β-ocimene            | --                       | 0.89| --    |
| 1,059 trans-β-ocimene          | --                       | 0.56| --    |
| 1,060 G-terpinene              | 2.95                     | --  | --    |
| 1,070 γ-terpinene              | --                       | 0.58| --    |
| 1,091 Terpinolene              | 3.38                     | --  | --    |
| 1,101 Linalool                 | 4                        | 14.68| --    |
| 1,102 2-Methylbutyl 2-methylbutyrate | --                     | 0.29| --    |
| 1,125 Cis-p-menth-2-en-2-1-ol  | 2.56                     | --  | --    |
| 1,128 cis-Menth-2-en-1-ol      | --                       | --  | 0.68  |
| 1,150 Cis-β-terpineol          | 0.27                     | --  | --    |
| 1,151 Lilac aldehyde D         | --                       | 1.06| --    |
| 1,163 Trans-β-terpineol        | 0.44                     | --  | --    |
| 1,166 Terpineol <3->            | --                       | --  | 1.17  |
| 1,172 terpinen-4-ol            | 9.21                     | 0.76| --    |
| 1,183 neo-dihydro carveol      | --                       | 1.35| --    |
| 1,187 α-terpineol              | 6.01                     | --  | --    |
| 1,196 Methyl salicylate        | --                       | 0.89| --    |
| 1,196 Cis-Piperitol            | 0.83                     | --  | --    |
| 1,252 Piperitone               | 0.56                     | --  | --    |
| 1,270 Cinnamonaldehyde <(E)>  | --                       | --  | 0.15  |
| 1,287 Bornyl acetate           | 0.13                     | --  | --    |
| 1,299 2-cis-dihydro acétate de tepinyl | 0.2               | --  | --    |
| 1,315 Déc-a-(2E,4E)-dien-1-ol   | 0.18                     | --  | --    |
| 1,322 Hexyl 2-methyl-3-pentenoat | --                     | --  | 0.48  |
| 1,352 α-acétate-terpinyl       | 2.18                     | --  | --    |
| 1,363 Cyclosativene            | 1.32                     | --  | --    |
| 1,364 Anisaldehyde <dirmethylacetalp-> | --                 | --  | 0.97  |

**Sesquiterpenes**

**hydrocarbonated Sesquiterpenes**

| Compound                        | 0.13       | --  | --    |
|--------------------------------|------------|-----|-------|
| 1,383 Longifolene               | 0.13       | --  | --    |
| 1,384 β-Bourbonene              | --         | 0.57| --    |
| 1,388 α-copaene                 | --         | 5.62|       |
| 1,399 p-Elemene                 | 0.05       | --  | --    |
| 1,416 β-Caryophyllene           | --         | 20.37| 0.72 |
| 1,427 (E)-Caryophyllene         | --         | --  | 0.69  |
| 1,430 β-Copaene                 | 0.46       | --  | 2.63  |
| 1,442 (+)-Aromandrene           | --         | 0.28|       |
| 1,455 α-humulene                | 0.57       | --  | 0.28  |
| 1,474 G-murolene                | 1.59       | --  | --    |
| 1,485 D-germacrene              | 0.51       | 8.79| 0.68  |
| 1,489 α-selinene                | --         | 1.39|       |
| 1,494 Bicyclogermacrene         | --         | 2.22|       |
| 1,495 Trans-β-guaiene           | --         | 0.7  |       |
| 1,500 β-isobolene               | --         | ----| 1.17  |
| 1,508 β-germacrene              | 1.61       | 4.7 |       |
| 1,509 γ-Cadinene                | --         | 2.35|       |
| 1,519 Nookatene                 | --         | 1.06|       |
| 1,524 Δ-cadinene                | 1.42       | --  | --    |
| 1,524 δ-Cadinene                | 0.3        | 0.28| 0.46  |
| 1,532 Danae ether               | --         | 0.21| --    |
| 1,533 α-calacorene              | --         | 0.21|       |
| 1,540 trans-Cadina-1,4-diene    | --         | 1.49|       |
| 1,563 7-Hydroxyfarneose         | --         | 0.8 | --    |
| 1,564 α-Cadinène                | 0.16       | --  | --    |

**Oxygenated Sesquiterpenes**

| Compound                        | 2.94       | --  | --    |
|--------------------------------|------------|-----|-------|
| 1,517 Cubebol                   | --         | --  | 2.94  |
| 1,532 10-epi-Cubebol            | 2.7        | --  | --    |
| 1,568 (E)-Nerolidol             | --         | --  | 1.96  |
| 1,569 Longipinanol              | --         | 3.65| --    |
| 1,592 viridilolor               | --         | 0.23| --    |
| 1,595 Fokienol                  | --         | 0.28| --    |
| 1,600 Oxyde de caryophyllène    | --         | 0.31|       |
According to Pellecuer of present in Psidium littorale camara is thought to be due to Cubebol. This molecule is compared to those of Cupressus macrocarpa; high toxicity shown by the essential oil of Cupressus macrocarpa s.l. However, the level of effectiveness Anopheles gambiae Lantana camara s.l. strain Anopheles gambiae and the site of collection of the larvae seems to depend on the plant species, the concentration used for the essential oils noted in our study larvicidal properties against insects [51, 52, 53]. However, the monoterpenes have long been recognised for their proven larvicidal activity of the essential oils but is absent in those of Cupressus macrocarpa and Lantana camara. The work of Hui-Jing et al. [54] highlighted the role played by this molecule in its pure state when evaluating the insecticidal activity of the ethanolic extract of Cryptomeria japonica on larvae of Aedes albopictus and Aedes aegypti. Furthermore, our results show that, for certain concentrations and for the same plant species, mean mortality numbers were significantly different for An. gambiae larvae from different collection sites (Tables 3 and 4). Thus, larvae collected in Yassa were more sensitive to essential oils than those collected in Youpwé. This result could be explained by the fact that Anopheles gambiae s.l. is a species complex [55, 56]. Studies have shown that of the species in the complex, An. coluzzii is the one that has developed the most adaptive characteristics to pollutants in poorly urbanised neighbourhoods in African cities, whereas An. gambiae s.s prefers peripheral neighbourhoods where the environment is still relatively natural [56, 57]. It should therefore be suggested that the strain collected in Yassa that is more sensitive to essential oils would be An. gambiae s.s, while the less sensitive strain from Youpwe would be An. coluzzii. However, a molecular analysis should be carried out to confirm this hypothesis.

### Biological Tests

**Larvicidal tests**

Larvicidal tests have shown that the essential oils of the leaves of Cupressus macrocarpa, Psidium littorale and Lantana camara have important biological properties against Anopheles gambiae s.l. However, the level of effectiveness seems to depend on the plant species, the concentration used and the site of collection of the Anopheles gambiae s.l. strain (Tables 3,4). LC50 and LC95 values determined from Henry's simplified table were used to classify essential oils according to their toxicity level on An. gambiae s.l. larvae. Essential oil of Psidium littorale was the most effective, followed by those of Lantana camara and Cupressus macrocarpa (table 4). According to Pellicer et al. [190], the toxicity of an essential oil is strongly related to its chemical composition. Thus, the larvicidal activity of the essential oils noted in our study would be due to their high monoterpene content. Monoterpenes have long been recognised for their proven larvicidal properties against insects [51, 52, 53]. However, the high toxicity shown by the essential oil of Psidium littorale compared to those of Cupressus macrocarpa and Lantana camara is thought to be due to Cubebol. This molecule is present in Psidium littorale oil but is absent in those of Cupressus macrocarpa and Lantana camara.

#### Table 3: Sensitivity of mature larvae of Anopheles gambiae s. l. to different concentrations of essential oils of Psidium littorale, Lantana camara and Cupressus macrocarpa, after 10 hours of exposure (Kruskal Wallis H-test and Mann-Whitney Z-test, P<0.05).

| Essential oils            | Concentration (ppm) | Mature larvae | Z-test | P-value |
|---------------------------|---------------------|---------------|--------|---------|
| Psidium littorale         |                     |               |        |         |
|                           | 150                 | Yassa         | 0.5127 |         |
|                           |                     | Youpwe        | -0.655 |         |
|                           | 100                 | Yassa         | -1.091 | 0.2752  |
|                           |                     | Youpwe        | -1.091 | 0.2752  |
|                           | 50                  | Yassa         | 0.202  | 0.0273* |
|                           |                     | Youpwe        | 0.202  | 0.0273* |
|                           | H-test              | Yassa         | --     | --      |
|                           |                     | Youpwe        | --     | --      |
|                           | P-value             | Yassa         | 0.368  | --      |
|                           |                     | Youpwe        | 0.0273*| --      |
| Lantana camara            |                     |               |        |         |
|                           | 150                 | Yassa         | 0.1266 |         |
|                           |                     | Youpwe        | 0.1266 |         |
|                           | 100                 | Yassa         | 0.0495 |         |
|                           |                     | Youpwe        | 0.0495 |         |
|                           | 50                  | Yassa         | 0.0495 |         |
|                           |                     | Youpwe        | 0.0495 |         |
|                           | H-test              | Yassa         | --     | --      |
|                           |                     | Youpwe        | --     | --      |
|                           | P-value             | Yassa         | 0.444  | --      |
|                           |                     | Youpwe        | 0.0273*| --      |
| Cupressus macrocarpa      |                     |               |        |         |
|                           | 150                 | Yassa         | 0.1676 |         |
|                           |                     | Youpwe        | 0.0495 |         |
|                           | 100                 | Yassa         | 0.0495 |         |
|                           |                     | Youpwe        | 0.0495 |         |
|                           | 50                  | Yassa         | 0.0495 |         |
|                           |                     | Youpwe        | 0.0495 |         |
|                           | H-test              | Yassa         | --     | --      |
|                           |                     | Youpwe        | --     | --      |
|                           | P-value             | Yassa         | 0.060  | 0.045*  |
|                           |                     | Youpwe        | 0.060  | 0.045*  |

*Statistically significant
Table 4: Lethal doses of essential oils capable of causing 50% and 95% mortality of mature larvae collected in the Youpwe and Yassa districts (Douala).

| Plants                | DL50  | DL95  |
|-----------------------|-------|-------|
|                       | Yassa | Youpwe| Yassa | Youpwe|
| Cupressus macrocarpa  | 60.445| 125.395| 170.827| 432.78 |
| Lantana camara        | 25.088| 83.657| 167.177| 250.939|
| Psidium littorale     | 7.337 | 49.275| 97.897 | 88.407 |

Antiplasmodial test
The essential oils of the three plants have shown some in vitro antiplasmodial activity against Plasmodium falciparum. However, the essential oil of Lantana camara (IC50=12.34 ppm) appears to be the most active, followed by that of Psidium littorale (IC50=115.455 ppm) and Cupressus macrocarpa (IC50=147.29 ppm) (figure 1). The strong antiplasmodial activity could be related to the high Linalol content of Lantana camara essential oil (14.68%). Terpenes such as farnesol, nerolidol, limonene, and linalol are known for in vitro inhibition of the biosynthesis of dolichol in the trophozoite and schizontal cycle of P. falciparum. Terpenes also have the ability to inhibit the biosynthesis of the isoprenic side chain of benzoquinone in the schizogonic cycle [58, 59, 60]. Based on their IC50 values, essential oil of Lantana camara has a moderate in vitro toxicity against Plasmodium falciparum, whereas those of Cupressus macrocarpa and Psidium littorale show no significant activity.

Conclusion
The present study has shown that Psidium littorale and Lantana camara have important larvicidal properties against Anopheles gambiae s.l. while Lantana camara has moderate activity against P. falciparum in vitro. These plants can therefore be considered as a source of new molecules against malaria germs and vectors.

Acknowledgements: The authors would like to thank the inhabitants of Yassa and Youpwe for their collaboration.

References
1. Carnevale et Robert. Les anophèles Biologie, transmission du Plasmodium et lutte antivectorielle. IRD Editions, collection didactique 2009,402.
2. WHO Malaria 2020. https://www.who.int/news-room/fact-sheets/detail/malaria. 09 November 2020.
3. WHO. 2017. Global response to malaria at crossroads. https://www.who.int/fr/news-room/detail/29-11-2017-global-response-to-malaria-at-crossroads. 17 janvier 2020.
4. Chandre F, Darriet F, Manguin S, Brengues C, Carnevalle P, Guillet P. Pyrethroid cross resistance spectrum among populations of Anopheles gambiae S.S. from Cote d’Ivoire. Journal of the American Mosquito Control Association 1999;15:53-59.
5. Diabate A, Baldet T, Chandre F, Akogbeto M, Guiguemde TR, Darriet F et al. The role of agricultural use of insecticides in resistance to pyrethroids in Anopheles gambiae s.l. in Burkina Faso. The American Journal of Tropical Medicine and Hygiene 2002;67:617-622.
6. Aikpon R, Agossa F, Osse R, Oussou O, Aizoun N, Oke-Agbo F et al. Resistance in Anopheles gambiae s.l. populations from Atacora department in Benin, West Africa: a threat for malaria vector control. Parasit Vectors 2013;6:192-198.
7. Ahoua ALP, Koffi AA, Adjia MA, Assi SB, Kouassi PK, N’Guessan R. Status of pyrethroid resistance in Anopheles gambiae s.l. populations in Southwestern Nigeria. Malaria Journal 2008;7:189-200.
8. Awolola T, Brooke BD, Hunt RH, Coetzee M, M zeakis P, Walliker D. Evidence of increasing Leu-Phe knockdown resistance mutation in Anopheles gambiae from Nigeria following a nationwide long-lasting insecticide-treated nets implementation. Malaria Journal 2002;96:849-852.
9. Overgaard HJ, Reddy VP, Abaga S, Matias A, Reddy MR, Kulkarni V et al. Malaria transmission after five years of vector control on Bioko Island, Equatorial Guinea. Parasit Vectors 2012;5:253-266.
11. Etang J, Chandre F, Guillet P, Manga L. Reduced bio-
efficacy of permethrin EC impregnated bednets against an Anopheles gambiae strain with oxidase-based 
pyrethroid tolerance. Malaria Journal 2004;3:46-52.

12. Nwane P, Etang J, Chouaibou M, Toto J, Koffi A, 
Mimpfoundi R. Multiple insecticide resistance 
mechanisms in Anopheles gambiae s.l. populations from 
Cameroon, Central Africa. Parasit Vectors 2013;6:41-54.

13. Pradines B, Dormoi J, Briolant S, Bogreau H. La 
résistance aux antipaludiques. Revue francophone des 
laboratoires 2010;422:51-62.

14. Tchoumboungang F, Dongmo PMJ, Sameza ML, Mbanjo 
EGN, Foko GBT, Zollo PHA et al. Larvicidal activity 
against Anopheles gambiae Giles and chemical composition of essential oils from four plants cultivated in 
Cameroon. Biotechnology, Agronomy, Society and 
Environment 2009;13(1):77-84.

15. Foko NP, Baldovini N, Moutay E, Mambu L, Belong P, 
Grellier P. Activity of Ocimum basilicum, Ocimum 
canum, and Cymbopogon citratus essential oils against 
Plasmodium falciparum and mature-stage larvae of 
Anopheles funestus S.S. Parasite 2014;21:33. doi: 
t10.1051/parasite/2014033. Epub 2014 Jul 7

16. Akono NP, Jazet DPM, Tonga C, Mache NP, Kekeounou 
S. Evaluation de la toxicité des huiles essentielles des 
feuilles de Callistemon citrinus, Cinnamomum 
zeylanicum et Psidium guajava sur les adultes d’Anopheles coluzzii Coetzeet et Wilkerson 2013, vecteur du paludisme urbain au Cameroun. Cameroon Journal of 
Biological and Biochemical Sciences 2017;25:12-21.

17. Foko G, Tamesse J, Messi J. Insecticidal effects of 
Capsicum annuum on Anopheles gambiae Giles under 
laboratory conditions. Journal of Entomology 
2007;4(4):299-307.

18. Ndome A, Tapondjou L, Tendonkeng F, Mbiop W. T 
evaluation des propriétés insecticides des feuilles de 
Callistemon viminalis (Myrtaceae) contre les adultes d’Acanthoscelides obtectus (Say) (Coleoptera; 
Bruchidae). Tropicultura 2009;27(3):137-143.

19. Boyom FF, Ngouana V, Zollo PH, Menut C, Bessiere 
JM, Gut J et al. Composition and anti-plasmodial 
activities of essential oils from some Cameroonien 
medicinal plants. Phytochemistry 2007;64(7):1269-1275.

20. Lucia A, Licastro S, Zerba E, Audino PG, Masuh H. 
Sensitivity of Aedes aegypti adults (Diptera: Culicidae) to 
the vapors of Eucalyptus essential oils. Bioresource 
technology 2009;100(23):6083-6087.

21. Mpendo EM, Dieng SD, Pouha M. Etude 
ethnobotanique des plantes médicinales utilisées dans le 
département du Haut-Nkam (Sud Cameroun). 
International Journal of Innovation and Applied Studies 
2017;21(4):574-595.

22. Adams RP. Identification of essential oils by gas 
chromatography quadrupole mass spectroscopy. Carol 
Stream, IL, USA: Allured Publishing Corporation 
2001,469.

23. Service MW. Mosquito Ecology: Field sampling 
methods, 2nd edition, Elsevier, Amplied Science 
Publisher, London 1993,988.

24. Desfontaines M, Tchikangwa I, Le Goff G, Robert V, 
Carnevale P. Influence de l’alimentation des larves d’Anopheles gambiae (Diptera, Culicidae) sur le 
développement préimaginal en insectarium. Bulletin de 
départemental et de documentation de l’O.C.E.A.C 1991;98:12- 
14.

25. Gillies MT, Coetzee M. A Supplement to the Anophelei 
afrique South of the Sahara. (Afrthropical Region) Publications of the South African Institute for 
Medical Research 1987;55:1-143.

26. Gillies MT, De Meillon B. The Anophelesi of Africa 
south of the Sahara (Ethiopian Zoogeographical Region). 
Publications of the South African Institute for Medical 
Research 1968;54:343.

27. Trager W, Jensen JB. Human malaria parasites in 
continuous culture. Science 1976;193:673-675.

28. WHO (World Health Organization). Bioassay method 
for the titration of Bacillus sphaericus: consultation on the 
development of Bacillus sphaericus as a microbial 
larvicide. World Health Organization 1985;3:85-95.

29. Desjardins RE, Graic JC, David H, Jeffrey DC. 
Quantitative assessment of antimalarial activity in vitro 
by a semi-automated micro-dilution technique. Antimicrobial Agents and Chemotherapy 1979;16:710- 
718. [PMC free article] (PubMed) [Google Scholar]

30. Guillou J, Moreau S, Moutay E, Sinou V, Forfar I, 
Belisle-Fabre S et al. New ferrocenic pyrrolo [1,2-a] 
quinoxaline derivatives: synthesis, and in vitro 
antimalarial activity. Bioorganic & Medicinal Chemistry 
2008;16:9133-9144.

31. Frontier S, Davout D, Gentilhomme V, Langadeuc Y. 
Statistiques pour les sciences de la vie et de l’environnement: Cours et exercices corrigés. Dunod, 
Paris 2001,410.

32. Sousa EO, Barreto FSS, Rodrigues FFG, Campos AR 
& Costa JGM. Chemical composition of the essential oils of Lantana camara L. and Lantana montevidensis. 
Journal of Essential Oil Research 2012;24:447-452.

33. Lida BP, Medeiro, Márcio dos S, Rocha, Sidney Gde L, 
Gustavo Rde S et al. Chemical constituents and 
evaluation of cytotoxic and antifungal activity of Lantana 
camara essential oils. Revista Brasileira de 
Farmacognosia Brazilian Journal of Pharmacognosy 
2012;22(6):1259-1267.

34. Nacira A, et Yousra B. Activité Antimicrobienne de 
l’Huile Essentielle du Cyprès Vert (Cupressus 
macrocarpa L’). Algerian Journal of Natural Products 
2017(5):455-462.

35. Vieira RF, Simon JE. Chemical Characterization of Basil 
(Ocimum spp.) Found in the Markets and Used in 
Traditional Medicine in Brazil Economic Botany. 
Published By: Springer 2000,207-216

36. Malizia RA, Cardell DA, Molli JS, González S, Guerra 
PE, Grau RJ. Volatile constituents of leaf oils from the 
Cupressaceae family: Part I. Cupressus macrocarpa 
Hartw C. Arizonian Greene and C. torulosa Don species 
growing in Argentina. Journal of Essential Oil Research 
2000;12:59-63.

37. Adams RP. Geographic variation in the leaf essential oils 
of Hesperocyparis (cupressus) abramsiana, H. goveniana 
and H. macrocarpa: systematic implications. Phytologia 
2009;91(1):226-243.

38. Giatropoulos A, Pitarokili D, Papaiannou F, 
Papachristos DP, Koliopoulos G, Manonoulou N et al. 
Essential oil composition, adult repellency and larvicidal 
activity of eight Cupressaceae species from Greece 
against Aedes albopictus (Diptera: Culicidae). Parasitology 
Research 2013;112:1113-112.
Lantana camara Linn. Growing in Brazil Northeastern. Academy of Sciences, Université de Douala. PO Box: 067 Dschang, Cameroon.

Patrick Akono Ntonga,
Laboratory of Animal Biology and Phsyiology, Faculty of Science, Université de Douala. PO Box: 24 157 Douala, Cameroon

Henri Gabriel Tsiila,
Biology and Applied Ecology Research Unit, Faculty of Science, University of Dschang, PO Box: 067 Dschang, Cameroon

Calvin Tonga,
Laboratory of Animal Biology and Phsyiology, Faculty of Science, Université de Douala. PO Box: 24 157 Douala, Cameroon

Pasma Mache Nkouandou
Laboratory of Animal Biology and Phsyiology, Faculty of Science, Université de Douala. PO Box: 24 157 Douala, Cameroon

Authors Name
Gaëlle Magne Tamdem,
Laboratory of Animal Biology and Phsyiology, Faculty of Science, Université de Douala. PO Box: 24 157 Douala, Cameroon
Christelle Awansi Djeukam  
Laboratory of Animal Biology and Physiology, Faculty of Science, Université de Douala. PO Box. 24 157 Douala, Cameroon

Rachel Ngaha  
Laboratory of Animal Biology and Physiology, Faculty of Science, Université de Douala. PO Box. 24 157 Douala, Cameroon

Odette Etoile Ngo Hondt  
Laboratory of Animal Biology and Physiology, Faculty of Science, Université de Douala. PO Box. 24 157 Douala, Cameroon

Romeo Mbongue  
Laboratory of Animal Biology and Physiology, Faculty of Science, Université de Douala. PO Box. 24 157 Douala, Cameroon

Willy Teukam Soh  
Laboratory of Animal Biology and Physiology, Faculty of Science, Université de Douala. PO Box. 24 157 Douala, Cameroon

Ronny Kojom Kamga  
Laboratory of Animal Biology and Physiology, Faculty of Science, Université de Douala. PO Box. 24 157 Douala, Cameroon

Loic Kojom Foko  
Laboratory of Animal Biology and Physiology, Faculty of Science, Université de Douala. PO Box. 24 157 Douala, Cameroon

Pierre Michel Jazet Dongmo  
Laboratory of Biochemistry, Faculty of Science, Université de Douala. PO Box. 24 157 Douala, Cameroon

Chantal Menut  
IBMM, Montpellier-UMR 5247, Faculty of Pharmacy, 15 Avenue Charles Flahault, 34093, Montpellier, France