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Evaluation of the insecticidal effect of acetonic extracts of the bark and leaves of *Boswellia dalzielii* Hutch (Burseraceae) on adults of *Anopheles gambiae* in Maroua, Far North Region Cameroon

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Abstract
The insecticidal effect of extracts from the bark and leaves of *B. dalzielii* was tested on adults of *A. gambiae*. A series of five mass concentrations of active material was prepared: 0.5 g/m^2 ; 1.5 g/m^2 ; 2.5 g/m^2; 3.5 g/m^2 and 5 g/m^2. For each concentration, 20 adult anopheles were introduced into the cone - WHO. The lethal concentration 50 (LC_{50}) and the lethal hour 50 (LH_{50}) of the extracts were determined. The LC_{50s} of *B. dalzielii* were 3.05 g/m^2 and 3.94 g/m^2 respectively for bark and leaves. The LH_{50s} were 7 h 46 min 08 sec and 9 h 52 min 25 sec respectively for bark and leaves. The extracts of the bark showed a rather high toxicity compared to that of the leaves. However, a non - significant difference ($\alpha > 0.05$) was recorded between the toxicity of the two extracts. Long - acting Insecticide Impregnated Mosquito Net (LLIN) showed a low LH_{50} (2 h 11 min 13 sec) compared to those of the *B. dalzielii* extracts. The results of this article have shown that there are local ways to chase or to kill mosquitoes in Far North Region Cameroon.

Keywords: Insecticidal effect, extracts, *Boswellia dalzielii*, *Anopheles gambiae*, Maroua, Cameroon

1. Introduction
Africa remains the most affected continent with 90% of malaria cases yearly [1]. The mortality rate due to malaria remains high in Cameroon, with an average of 4,000 deaths in 2012 [1]. The most widely used vector control methods are based on impregnated mosquito nets, sprays and insecticides by fumigation [2]. One of the methods that demonstrated its effectiveness is the control of populations of vectors directly involved in malaria transmission [2]. Despite the progress of science, the scientific community faces many obstacles in the fight against malaria. These include increased resistance of *Plasmodium* spp to curative drugs, resistance of vectors to insecticides, high cost of synthetic products and toxicity to organisms and environment [3]. The search for new insecticides, biodegradable and better known to local communities is necessary [4]. An ethnobotanical survey revealed that many natural substances or their derivatives are commonly used as insecticides or insect repellents by the populations of Maroua and its surroundings [5]. Some plants contain in their structure substances which have insecticidal and insect repellents [6]. These plants can be used as alternative insecticides [7]. The general objective of this article is to contribute to the fight against malaria in the Far North Region Cameroon by reducing the populations of mosquito vectors of *Plasmodium* spp. The specific objectives are to improve the method of using these extracts by determining the lethal concentration 50 (LC_{50}) and the lethal hour 50 (LH_{50}) on adults of *A. gambiae*.

2. Materials and methods
2.1 Presentation of the study site
The study was conducted from April 15 to May 20, 2013 in the city of Maroua, the regional capital of Far North Cameroon. Maroua is a town (Fig. 1) of about 315,372 inhabitants according to the 2005 census. It is located at an altitude of 400 m, 10 ° 35 'north latitude and 14 ° 18' East longitude. It stretches along the banks of the Mayo - Kaliao [8]. The climate is of the tropical type with Sahelian tendency [9]. Annual rainfall is 700 - 800 mm^3 with an average temperature of 30 °C [9]. Vegetation is characteristic of dry Sudano - Sahelian savannas [10]. The hydrographic network is characterized by dry, temporary watercourses during most of the year (October - May).

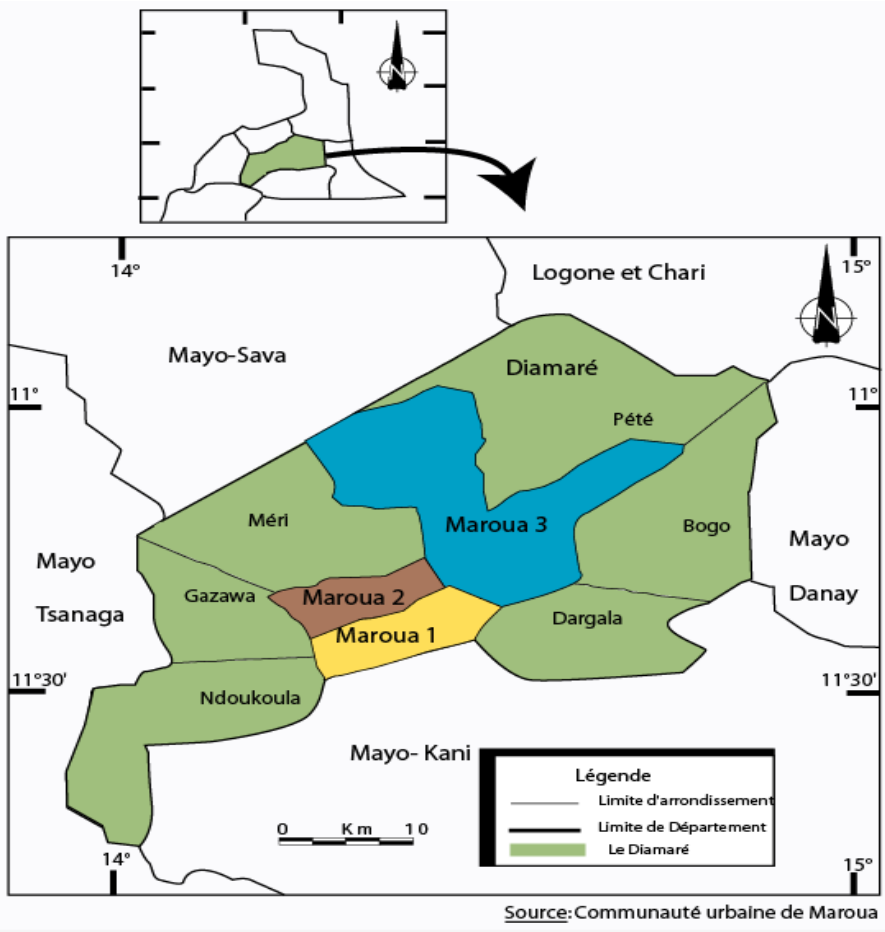

Fig 1: Far North Region Cameroon

2.2 Methodology

Boswellia dalzielii (Fig. 2) was selected at the end of an ethnobotanical survey among the populations of the Far North Region Cameroon [5]. The leaves (Fig. 3) and trunk bark (Fig. 4) of *B. dalzielii* were harvested from several individuals at the same time in March 2013 in Mouzourtouk, a locality located at 30 km south of Maroua. These two parts were then dried in the shade separately for 10 days and then crushed with a mortar in order to obtain the powders.

Fig 2: *B. dalzielii*

Fig 3: Leaves of *B. dalzielii*

Fig 4: Bark of *B. dalzielii*

2.2.1 Preparation of extracts of *Boswellia dalzielii*

The extraction of the active substance was carried out at the Laboratory of Organic Chemistry of the Higher Teachers' Training College of the University of Maroua. The method of extraction of the active ingredients is that described by [11]. The bark and leaves were previously subjected to mechanical treatments such as chopping, grating and grinding. This facilitates the transport of the materials and makes the active molecules much more extractable. The powders were weighed using a HCB123 electronic balance (Fig. 5) with a precision of 0.001 g. An amount of 500 g of each of the powders was macerated in 4L of pure acetone. The paste was homogenized by manual stirring and left to stand for 48 hours. This is the maceration whose purpose was to cause the release of the active molecules of the plant. Then, the macerate was filtered using Wattman paper placed in a funnel (Fig.6).

Fig 5: Weighing powders

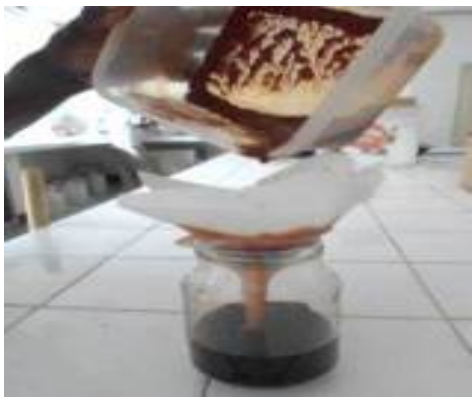

Fig 6: Filtration of the paste of leaves and bark

2.2.2 Concentration of extracts

The concentration allowed the acetone to be separated from the active molecules released from the leaf or bark powders. It consists in placing the mixture (extract + acetone) in a rotary evaporator (Fig. 7) set at a temperature of 50 °C. This temperature is close to the evaporation temperature of the acetone and allows a slow evaporation of the latter. Once the rotavapor is turned on, the concentration flask immersed in the Bain Marie and containing the vegetable extract starts to rotate. By turning, it lets the acetone escape in the form of vapor. These vapors are then conducted to a condenser. After cooling and condensation, the solvent is collected in the collecting flask and only the pure plant extract remains in the concentration flask which will serve as the active ingredient for the insecticidal effect tests. The yield of the extract was calculated by making the mass ratio of the pure extract obtained by the total mass of powder times 100, according to the formula below:

$$\text{Yield} = \frac{\text{Mass of pure extract}}{\text{Total mass of the powder}} \text{ X } 100$$

Fig 7: Concentration of Rota vapor extracts

2.2.3 Impregnation of mosquito net with extracts

Ranges of concentrations more or less similar to WHO standards were chosen for testing. The following active compound concentrations were set: 0.5 g/m^2; 1.5 g/m^2; 2.5 g/m^2; 3.5 g/m^2 and 5 g/m^2. Concentrations are the same for leaves and bark extracts. Using a pipette, the volume of 5 mL of hexane is taken and poured into a flask containing the mass of active material corresponding to a given concentration. After complete dissolution of the extract in a flask containing the hexane, the portions of mosquito net were introduced. The impregnated mosquito net portions were held in hermetically sealed flasks for fifteen minutes. This is done in order to adhere well the active material to the mosquito net. The cones used for the tests are those of the cone - WHO model [12] (Fig. 8). Each cone is then covered underneath by the impregnated mosquito net and plugged over by a piece of hydrophilic cotton. The portions of impregnated mosquito net are left to dry at room temperature for 1 hour in order to adhere the extract and completely evaporate the hexane

Fig 8: Cone – WHO

2.2.4 Collecting and breeding of *Anopheles gambiae* larvae and nymphs

Anopheles gambiae larvae were collected from the breeding site using dipping method [13] (Fig. 9) and were distributed in trays (Fig. 10); each tray contained 150 to 200 larvae; 15 trays at the rate of five per larval breeding were used for this purpose. The trays containing the breeding water then

received every other day 1 g of nutritive powder made of a mixture of equal quantities of shrimp (*Palaemon serratus:* Palaemonidae) and crushed biscuits of the "Parle - G" brand (fat: 78.2 g, dietary fiber: 12.3 g, protein: 0.6 g, calcium: 6.5 g, energy: 15 mg). One to two days after the distribution of the larvae in the trays, the nymphs appear. Subsequently, the nymphs were removed daily from the tray and introduced into the glasses. At the end of three to five days, adult mosquitoes appear.

Fig 9: Collection of *A. gambiae* larvae

Fig 10: *A. gambiae* larvae in tray for rearing

2.2.5. Test of the insecticidal effect of extracts of *Boswellia dalzielii*

Three sets of tests were carried out. Impregnated mosquito nets are secured under the cones with adhesive tape. Seven cones were used: five cone were reserved for different concentrations; one with the mosquito net impregnated with deltamethrin 55 mg/m^2 as a positive control; one wearing a mosquito net impregnated with hexane constituting the negative control. The mosquitoes tested were five to seven days old. A blood meal on a rabbit was administered 24 hours before the test in order to avoid the results bias that would result from hunger. Before the tests, the anopheles were observed for a duration of 1 h. Twenty anopheles are then introduced into each cone using a test tube (Fig. 11).

Fig 11: Introduction of *A. gambiae* in cones

Once inside, the opening of the cone is closed to prevent mosquitoes from coming out. A stopwatch was started to observe the behavior of the mosquitoes, the knock - down effect was observed after 3 min and the observations continued at 5 min intervals for 60 min. The observations continued for 1 hour for 10 hours and experimental device (Fig. 12) was maintained for 24 h and the mortality was evaluated. The test is thus repeated three times for each of the concentrations and for each of the plant extracts.

Fig. 12: Exposure of *A. gambiae* to extracts for 24 h

2.3 Identification of adult mosquitoes
The identification of adult anopheles has been confirmed in the Laboratory of Applied Entomology of the Department of Biological Sciences of the Faculty of Sciences of the University of Ngaoundere, Cameroon. The identification was done using a microscope, a binocular lens and morphological identification keys. The keys used are the keys of [14] and [15] for Anophelinae.

2.4 Data analysis
The analysis of the data was done using descriptive statistics to calculate averages, standard deviations and percentages. The *Z* test and Student *t* test were using to compare two averages. The formulas from [16] were using to calculate LC_{50} and HL_{50}. Mann - Whitney test *U* to compare independent variables and Excel software for 2007.

3. Results and discussion
3.1 Yield of extracts of *Boswellia dalzielii*
At the end of the extraction, masses of 67.14 g of bark and 45 g of leaves were obtained from 500 g of powder. This corresponds to the yields of 13.42% for the bark and 9.62% for the leaves. The bark fragments of *B. dalzielii* contain more active matter than the leaves. Certain authors [17] made an observation that extracts from different parts of the same plant or different plants; subjected to the same solvent gives different yields. According to [18] the active molecules are not uniformly distributed in plants.

3.2 Extracts effects on *Anopheles gambiae*
The experiment has shown that the control with the hexane - impregnated mosquito net had a mortality rate of 5% after 24 hours of exposure. The control at Long - Acting Insecticide Impregnated Mosquito Net (LLIN) induced a knock - down effect of 20% and then a very high mortality rate reaching 100% in 24 hours. For the extracts of the two parts of *B. dalzielii*, the mortality rate of *A. gambiae* increased as the duration of exposure and the concentration of extract increased. The active ingredient proportions of plant extracts are higher in the high concentrations than in the low ones [19]. No concentration of plant extract produced a knock - down effect. For both extracts, concentrations 0.5 g/m^2 and 1.5 g/m^2 induced no mortality before 60 min of exposure. Bark extracts at concentrations 2.5 g/m^2, 3.5 g/m^2 and 5 g/m^2 induced 65%, 80% and 100% mortality respectively in 24 h (Fig. 12). The extracts of the leaves showed a less effective insecticidal activity than that of the bark. The concentrations 2.5 g/m^2; 3.5 g/m^2 and 5 g/m^2 resulted in 55%, 75% and 95% mortality respectively in 24 h for leaf extracts (Fig. 13). It has been found that the insecticidal activity of plant extracts varies with the part used for the tests and the synergistic effect of these substances is at the root of its insecticidal effect [20]. Certain authors have proved that the insecticidal effect of the plant results from the toxic molecules stored in its cells [19]. This means that the extracts of the bark of *B. dalzielii* contain in their structures large quantities of odoriferous molecules. According to [19], the Burseraceae, including *B. dalzielii* are characterized anatomically and chemically by the presence of fragrant oligo - gommo - odorous secreting channels providing incense or oliban, myrrh, opopanax, elemi used in pharmacies for their balsamic properties. The essential oil of *B. dalzielii* is dominated by hydrogenated monoterpenes (63.9%), their oxygenated derivatives being much less abundant (3.9%) and sesquiterpenes also being a minority (6.5%) [21]. Moreover, it has been found that hydrocarbon monoterpenes are highly toxic molecules on closed mosquitoes and terpenoids (acetate bornyl and terpenolene) are adulticide for mosquitoes [22]. In Cameroon, it has been shown that the hydrocarbon monoterpenes and oxygenated sesquiterpenes have more effective insecticidal effect in insects [23].

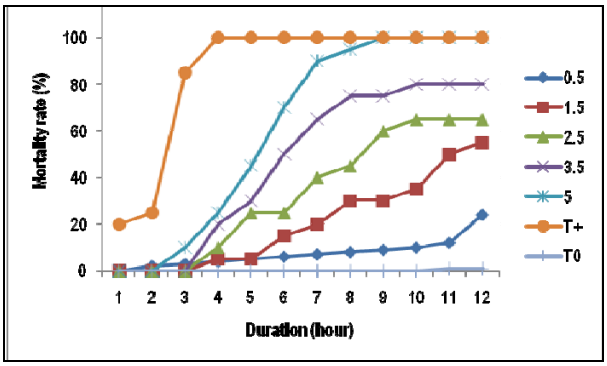

Fig 13: Mortality rate of *A. gambiae* to extracts of the bark of *B. dalzielii*

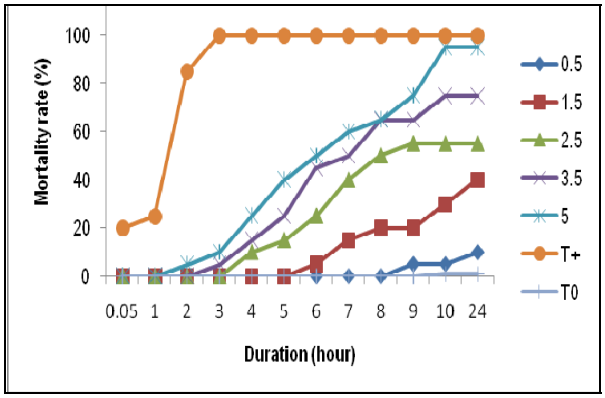

Fig 14: Anopheles mortality rate of *A. gambiae* with extracts from leaves of *B. dalzielii*

3.3 Lethal Concentration 50 (LC_{50})
The determination of the lethal concentration 50 (LC_{50}) of *B. dalzielii* extracts on *A. gambiae* was carried out using method [16] basing on the regression lines obtained by converting the percent probit mortality after 24 hours of exposure as a function of the decimal logarithm of concentrations. The extract of the bark of *B. dalzielii* gave an LC_{50} of 3.05 g/m^2, lower than that of its leaves (LC_{50} = 3.94 *B. dalzielii* contains saponins, alkaloids, flavonoids, tannins, resin, steroids and triterpenes, which are responsible for the insecticidal effect [24]. Mann – Whitney test revealed that there was no significant difference between the LC_{50}s of the two extracts ($\alpha > 0.05$).

3.4 Lethal Hours 50 (LH_{50})
The determination of the lethal hour 50 (LH_{50}) of *B. dalzielii* extracts on *A. gambiae* was made from the regression lines obtained by transformation of the probit mortality after 24 hours of exposure as a function of the decimal hour logarithm. The leaf hour 50 of the leaves of *B. dalzielii* is 9 h 52 min 25 sec, and that of the bark is 7 h 46 min 20 sec. Long - lasting impregnated mosquito nets (LLINs) presented a lethal hour 50 (LH_{50}) of 2 h 11 min 13 sec. The Mann - Whitney test revealed that there was no significant difference between the HL_{50}s of the leaf extracts and that of the *B. dalzielii* bark ($\alpha >$ 0.05). However, a very highly significant difference ($\alpha <$

0.001) was recorded between the LH_{50} of MILDA and those of the two extracts of *B. dalzielii*. The correlation between LC_{50} and LH_{50} is justified because the extracts of the bark showed an LC_{50} and LH_{50} lower than those of the leaves. These results corroborate those of [25] who showed that the bark of *B. dalziellii* trunk contains more toxic substances than the leaves. Biological tests were carried out by [26] and these authors have proved that the essential oils of some local plants also have larvicidal properties in *A. gambiae*, including *Cymbopogon citratus*, *Thymus vulgaris*, *Ocimum gratissimum* and *Ocimum canum*, which induced 100% larval mortality of stage 4 of *A. gambiae* at respective concentrations of 100 ppm, 200 ppm, 350 ppm and 400 ppm. A study was conducted on the efficacy of six essential oils extracted from local plants in northern Cameroon on *A. gambiae* Giles 1902 and found HL_{50}s between 6 h 36 min 36 sec (*Ocimum canum*) and 14 h 22 min 10 sec (*Eucalyptus camaldulensis*) [27]. Hydrocarbon monoterpenes, molecules contained in plants are very toxic in closed mosquitoes [22].

4. Conclusion

Assessments of the insecticidal effect of *B. dalzielii* on adults of *A. gambiae* revealed that the flora of the Far North Region Cameroon has a potential for vector control. The bark of *B. dalzielii* showed an LC_{50} of 3.05 g/m^2 while that of the leaves was 3.94 g/m^2. The LH_{50}s are 7 h 46 min 08 sec and 9 h 52 min 25 sec respectively for barks and leaves. Given the extremely high level of poverty in this area, valorization of natural resources would be an alternative for malaria control. It would be wise to extend the study to other plant species presumed to have insecticidal effects.

5. Thanks

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