International Journal of Engineering Research and General Science Volume 4, Issue 2, March-April, 2016
ISSN 2091-2730

Repellent activity of essential oil extracted from *Artemisia annua* (Asteracea) grown in Cameroon (Africa) and Luxembourg (Europe)

Nkuitchou-Chougouo K. Rosine D.[1, 4], Kouamouo Jonas[1], Wannang N. Noel[2], Yopa K. Nelly[1], Titilayo O. Johnson[2] Sandjon Bertrand[3], Lutgen Pierre[4], Tane Pierre[4], Moyou S. Roger[5].

[1]Pharmacy Branch, Faculty of Health Sciences, Université des Montagnes, Bangangté Cameroon P.O.Box 208

[2]African Centre of Excellence in Phytomedecine Research and Development, University of Jos Nigeria

[3]Phytorica Laboratory, Pharmacy Bonapriso, Njoh Njoh – street, Douala Cameroon

[4]Iwerliewen Fir Bedreete Volleker (IFBV)-BELHERB, BP 98 Niederanven, L-6905 Luxembourg

[5]Laboratory of Natural Products, Department of Organic Chemistry, University of Dschang, Cameroon

[6] Parasitology Laboratory, Institute of Medical Research and the Study of Medicinal Plants (IMPM) Yaoundé Cameroon

Email: nrosinedesiree12@yahoo.com; tel: +237 677468510

Introduction: *Artemisia annua* is an annual plant of China origin well known for its medicinal value due to its diverse chemical composition. The extract of *A. annua* cultivated in Cameroon is rich in essential oil; the larvicidal antibacterial and antifungal properties of *A. annua* had been reported. In the continuation of these works, we investigated the repulsive effect of this plant material.

Materials and methods: The essential oil of *Artemisia annua* of Cameroon and that of Luxemburg were obtained by hydro-distillation of dry leaves of *Artemisia annua* from the two countries. In order to determine their repulsive activities, two methods were used: One of the standard W.H.O (night catch on exposed human legs) and Cage tests (Bigoga models). All methods were validated and the results analyzed using student *t* test.

***Results*:** The turnover of the extraction of the two essential oils gave 0.16%. The repulsive activity was evaluated at the concentration of 0, 50, 100, 200, 300 and 400 part per million (ppm). After the application of the two types of oil by the two methods, we observed that the two plants produced repulsive properties from 50 ppm up to 400 ppm. The time of protection is in an average of 3 hours, but this reduces as the essential oil is diluted.

***Conclusion*:** Essential oil extract from the leaves of *Artemisia annua* cultivated in Cameroon and in Luxemburg have similar repulsive properties on adult *Anopheles gambiae*.

Key words: *Artemisia annua*, *Anopheles gambiae*, essential oil, repulsive, hydro-distillation

INTRODUCTION

Malaria is an infectious disease caused by a parasite of the genus *Plasmodium*, which infects red blood cells. The disease is as old as history and till date, it is a major problem especially in Asia, Africa, Central America, South America and the Caribbean. It remains

the most important parasitic disease affecting mostly children under 5 years and pregnant women [1]. According to WHO, (2015) an estimate of 214 million (with an uncertainty range between 154 million and 289 million) were reported cases of malaria, with about 438,000 (with a margin of uncertainty between 390000 and 636000) deaths [2]. Approximately 3.2 billion people, almost half the world's population, are exposed to malaria risk. This high rate is alarming, thus, scientists around the world have taken from their health priorities the fight against malaria. Recently, Niyondiko and Dembélé introduced soap for protection against the vector [3]. This innovation reinforces the curative control measures (such as ACTs) and preventive (such as bed nets impregnated with long-term consolidation of the domestic environment, sprays, repellants etc) in order to minimize the burden of malaria.

An ethnobotanical study conducted in Kilimanjaro (Tanzania) showed that the most widely used repellent, by the locals are *Ocimum kilimandscharicum* and *Ocimum suave*. The study further showed that the essential oils in these plant material is responsible for activity against bites and perching activity from Anopheles vectors [1].

Much work has been done on *Artemisia annua* in the laboratories of the (Université des Montagnes), and it showed that it is very rich in chemical compounds giving it numerous anti malarial, larvicidal, antibacterial and antifungal properties [4]. So far, positive results have been obtained on the curative plan, but further efforts are still required in terms of prevention.

Optimum protection can be provided by its application as a repellent. It is in this context of continual fight against malaria that we studied the repellant activity of essential oils extracted from *A. annua*.

MATERIALS AND METHODS

Study Sites

The extraction of essential oil from *A. annua* (Cameroon and Luxembourg) was conducted in Phytorica laboratory in Douala (Dr. Bertrand Sandjon) located at the Njoh Njoh - street, with a hydro- distillation device. The study of repellant properties was conducted at night, in the locality of Ebang, so chosen because of the vegetation and climatic condition which favours mosquito growth (10 km from the city of Yaounde on the Yaounde - Obala road in Cameroon) and in the premises of the Parasitology laboratory, in the Institute of Medical Research and the Study of Medicinal Plants (IMPM) in Yaoundé (central region).

Plant Material

A. annua was registered at the Cameroon National Herbarium with voucher number 65647/HNC. Samples used in this study were grown in September 2012 and harvested in February 2013. The

fresh leaves were used.

Extraction of Essential oil

The principle of hydro-distillation was used [5]. 200g of fresh leaves was boiled in 1 L water in a 2L flask for 2 hours. The steam drives essential oils from the product through a column connected to a condenser which condenses the vapor (Figure 1). The hydrosol obtained was then allowed to settle into a separatory funnel in which the previous mixture separates into two immiscible phases. The phase which contains the essential oil floats because it is of lower density than water. The oils extracted were dried using anhydrous sodium sulfate.

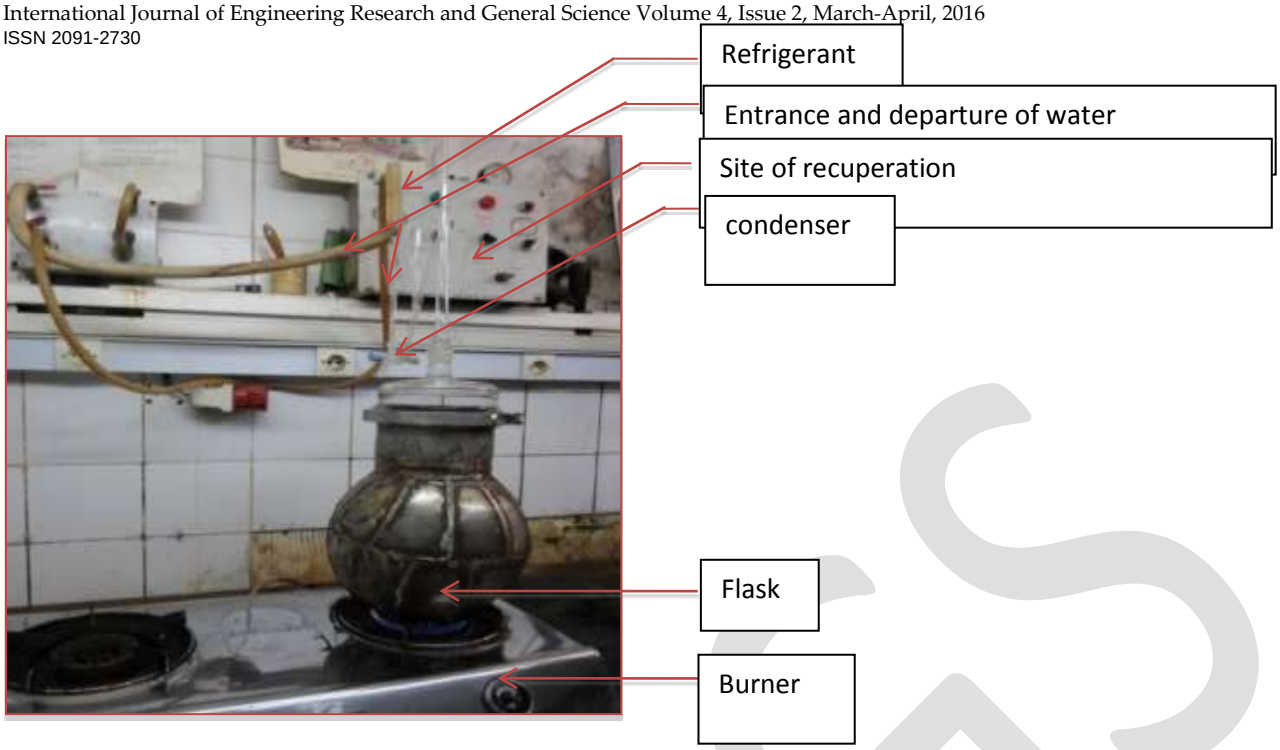


Figure 1: Scheme of Laboratory Phytorica Hydro-distillation

Culturing of Anopheles gambiae eggs

The *Anopheles gambiae* eggs were obtained from the insectarium of the Coordinating Organization for the fight Against Endemic Diseases in Central Africa (OCEAC) in Yaounde, Cameroon. This is basically to put in contact with water the *Anopheles* eggs so that they hatch into larvae that later become adult Anopheles. The eggs were placed in contact with the source water in plastic plates (3 cm^3 of water in plastic plates of 20 cm in diameter). Hatching occurred 48 hours later. Upon hatching, the larvae were fed with a mixture of proteins. The larvae were exposed to the sun during the day (from 8 to 16 hours) and set in the dark at night to observe the circadian cycle they need for their growth.

Breeding process of Anopheles

The embryonic development lasted two days and the egg was hatched to release an L1 larva measuring just 1 millimeter. These larvae of Anopheles were maintained parallel to the surface of the water. After consecutive molts, the larva reached the stage 4 (L4), measuring 6 to 12 mm. L4 larvae passed to the pupal stage. At the end of this stage, the nymph was immobilized to the surface of the water, the mosquito extended its abdomen and an adult mosquito emerged with a dorsal slit of the exoskeleton (Figure 2).

This emergence lasted only a few minutes though left for 3 days to mature (while feeding them with sugar water) prior to experiment of the test cage method.

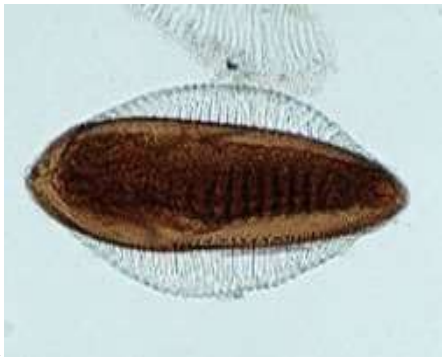
A  B  C 

Figure 2: A Eggs, B larvae and C pupae of Anopheles C [16]

We cultured 1500 Anopheles eggs; 1200 eggs (80%) have reached the end of their growth.

Repellant activity

This was achieved in 2 ways: through the night trapping method on human volunteers and by the test method in cages. To afford this, we obtain the inform consent of participants and ethical clearance N°: 2013/CIE-UdM/Pr was obtained from the ethical institutional comity of "Université des Montagnes".

The concentrations were made at 0, 50, 100, 200, 300 and 400 ppm by dilution with ethanol. Upon administration of the essential oil by rubbing on the legs of volunteers and observing the perch and bite activities of the vector from 8 pm to 6 am daily (figure3). This experiment was done in a quiet environment, to avoid distraction from noise.

Figure 3: Night trapping method on human volunteers

In the test cages methods, the test was based on observing Anopheles adults movements between an untreated cage and a cage pretreated with increasing concentrations of test material (figure 4).

Figure 4: Test cage of Bigoga model

The test was done under laboratory controlled temperature (27±2°C) and relative humidity (80±2%). A 1ml of formulation 50, 100, 200, 300 and 400 ppm of oil fraction was applied on the inner surfaces of which the surface is about 324 cm^2 (treated), and connected to the untreated group [6].

100 female Anopheles were introduced into the cages and mosquito behavior was observed every 30 minutes for three (3) days. The protection time was measured (period between the introduction of the mosquitoes in the treated cage and the time it takes to move to the untreated). If no mosquito returns to the cage treated after 4 hours, the test is stopped and the protection time is rated 4 hours. Each test is repeated 2 times.

RESULTS

Extraction and characteristics of essential oils

Four kilograms of dry leaves of *Artemisia annua* from both sources produced a yield of 0.16% yield (Table I). The characteristics were camphor smell, light green in color and have an oily consistency.

Table I: Extraction yield and characteristics of essential oils

	***Artemisia annua* from Cameroun**	***Artemisia annua* from Luxembourg**
Quantity of dry leaves	4kg	4kg
Weight of E.O	6.5g	6.5g
Yield	0.16%	0.16%
Color	Light green	Light green
Consistency	Oily	Oily
Odor	Aromatic camphor	Aromatic camphor

Repellent activity on volunteers

The harvest consists of 10% of Mansonia, Culex 60% and 30% *A. gambiae*. Treatment with extract (E.O) of Cameroon reduced *A. gambiae* to 4% and Mansoni to 1%. The majority population was Culex 95%. Treatment with extract of Luxembourg reduced *A. gambiae* to 25%, the Culex was (70%) (Table II)

Table II: **Summary of quantities of captured vectors**

	Control (witness)	E.O from Cameroun	E.O from Luxembourg
Mansonia	10%	1%	5%
Culex	60%	95%	70%
Anopheles gambiae	30%	4%	25%
Total	100%	100%	100%

Repellent activity in the laboratory

At concentrations of 50 and 100 ppm, the repellant effect of the two essential oils was observed from the 30th minute. This effect was maximal after 1 hour of exposure to 50 ppm. It is from 1:30 min to 100 ppm. This activity later decreased after 150 minutes.

After 4 hours of exposure, residual repellant effect was found to be 10% for Anopheles at 100 ppm. There was more repulsive effect at a dose of 50 ppm after 4 hours of exposure.

At 200 ppm, the repellant effect was immediately maximal, resulting in the abandonment of the cage treated with the extracts by all the vectors. This repulsive effect decreased slightly to 150 minutes (2:30 min). At 4 hours, the residual effect was 10%.

At 300 ppm, the protection was 100% at 3 hours. At 4 hours this rate of protection decreased to 30%

A concentration of 400 ppm, the total protection time was 3h 30min. At the fourth time, the repulsion rate was 90% (Figure 5 and 6)

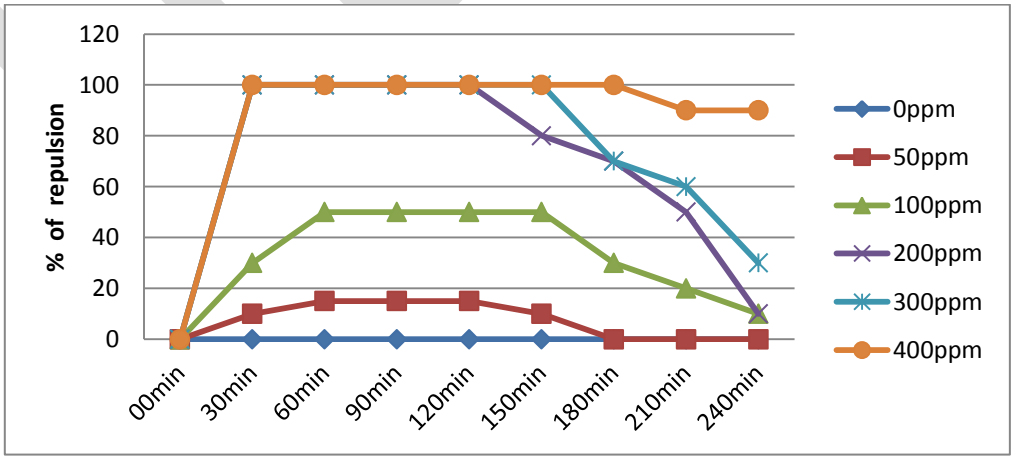


Figure 5: Repulsion of *A. gambiae* in the laboratory (Cameroon)

Luxembourg's essential oil repellant activity is the same as that of Cameroon (Figure 4)

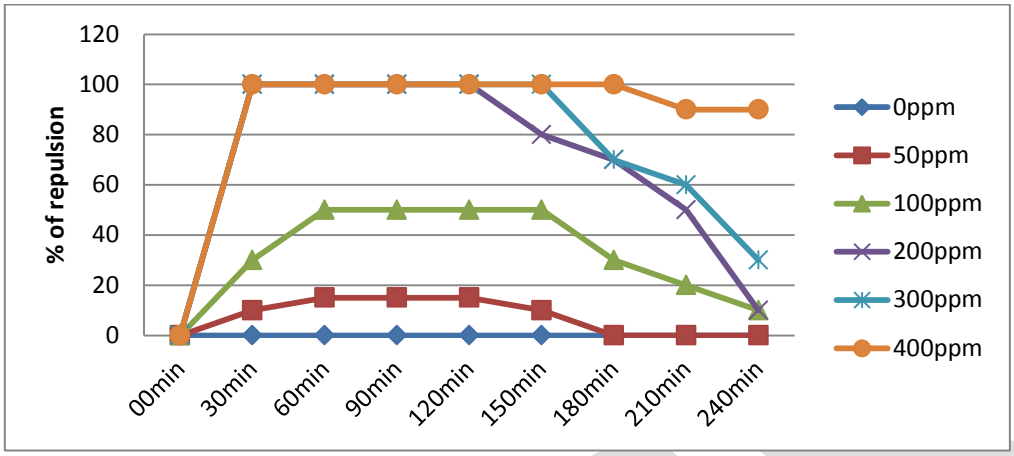


Figure 6: Repulsion of *A. gambiae* in the laboratory (Luxembourg)

DISCUSSION

Hydro- distillation extraction efficiency

The essential oil extracted produced a yield of 0.16 %. In general, the yield of essential oils from aromatic plants is very low. This is the case for example, rose essential oil which has an extraction yield comprised between 0.1 and 0.35% [7]. The yield we obtained in this case is comparable to that found by Valtcho et al in a study on the impact of time on the extraction efficiency of *A. annua* by steam distillation [8]. They obtained yields ranging from 0.05 to 0.35 % for an extraction time ranging from 0 to 240 min, the highest yield corresponding to the longer duration of extraction [8] and comparable performance 0.19% was obtained by Domum of Cameroon in 2012 [4].

Similar results were also cited by Namdeo et al, with yields ranging from 0.14 to 0.66 % of the hydro-distillation of dry leaves of *A. annua* [9]. Zewdinesh et al in turn obtained extraction yields ranging from 0.04 to 1.9%, by solvent extraction, while varying the harvest age and size of plants [10]. Higher yields than ours have been reported with other species of the genus Artemisia 1.1% was obtained for *A. absinthium* and 1.2% for *A. arborescens* [11].

Physical characteristics

The aromatic camphor smell of *A. annua* essential oil that we observed may be explained by their high content of camphor (50%) at least demonstrated by studies of Chougouo [12]. Their oily consistency and coloring, have allowed us to say that these two species have consistent characteristics [13]. Indeed, the European Pharmacopoeia specifies that any essential oil should be oily in consistency and sometimes colorful. Similarly, Domum described *A. annua* as oily consistency, with light green color and aromatic camphor smell, with a density of 0.90, an index 19.70 of acid, a saponification value of 25.00, an ester number of 5.30 and positive solubility in alcohol at 75 Åã and 95 Åã. which is in conformity with our results.

Repellent activity: Activity on volunteers

The chosen site-the town of Ebang (in forest areas) has an adverse weather condition to encourages mosquito growth. Commonly seen were the female Anopheles and other species, namely the Culex (which is the species most represented) and a small number of Mansonia. These three types are responsible for the transmission of various diseases. The *A. gambiae* transmits malaria and lymphatic filariasis, Culex transmits lymphatic filariasis, Japanese encephalitis and other viral diseases, Aedes transmits yellow fever, dengue and lymphatic filariasis and Mansonia transmits lymphatic filariasis [14]. Though this work has some ethical issues, especially when it involved human volunteers, our attention was drawn to a follow-up plan for the treatment of these volunteers.

Repellent activity: Laboratory activity

It is observed that the 2 essential oils possess similar repulsive properties and that the optimum concentration of both is 200 ppm. Protection time varies depending on the concentration used, but it is 2h30min from 200 ppm. Many farmers observed that the cultures of *A. annua* around the house were regress malaria infection, action that would be due to the presence of essential oils in the plant. This hypothesis was verified with our studies because we noticed a growing repulsion depending on the concentration (eg 200 ppm is a time of 2h30min protection, 300 ppm is a time of 3 hours and protection of 400 ppm has a protection time of 3h30min), especially since this repulsion can even explain that secondary metabolites of these plant may have repellant properties on certain insects [15].

This study demonstrated that there was a more marked activity on *A. gambiae*. This action is not noticeable on the Culex at the doses used. The work of Chougouo on the physicochemical characterization of *A. anuua* of Cameroon and Luxembourg have identified despite the chemical variability in ecosystem function, 13 volatile compounds that are: camphor, α-pinene, beta- pinene, 3-carene, the α-terpinene, limonene, eucalyptol, artemisia ketone, copaene, the camphene, caryophylène, menthol, and α -terpineol [12]. The differences between the 2 oils related to the amounts of these volatile compounds, scopoletin and artemisinin.

Indeed, analysis of species showed that *A. annua* grown in Cameroon is rich in camphor and menthol but less rich in artemisia ketone, eucalyptol, limonene, copaene than that grown in Luxembourg [16]. Studies have shown that camphor repels flies, mosquitoes, moths and most insects, however, geranium oil (Mainly of camphor) has a very strong repellency against mosquitoes [17, 18]. Which may be by analogy the same case for *A. annua* which is predominantly composed of camphor (50% of all volatile compounds). Also known as repellent a are menthol, camphene, eucalyptol, limonene and α -terpinene [6,18,19,20].

Studies have shown that other compounds, eg α-terpinene which is the major compound found in the leaves of *Chenopodium ambrosioides* Linn have repellant activity on *A. gambiae* [6]. Soybean also repels [21], so also is oil from *Syzygium aromaticum* (cloves) and *Zanthoxylum Limonella* (Makaen)[22]. Citronella oil (solution v/v 1% and 15% v/cream) has a repulsion activity attributed to citral, which is a major oil constituent [19] .The Neem cream provided good protection against *Aedes albopictus*, *Aedes aegypti*, *Culex quinquefasciats*, *Anopheles culcifacies* and *Anopheles subpictus.* The application of cream shows 78% (range 65-95) protection against Aedes, 89% (range 66-100) against Culex and 94.4 % (range 66-100) against Anopheles for 4 hours of protection time [23]. Repulsion rate obtained with DEET, chemical synthesis, is on average of 92 to 100 % for 7h protection time after application [24].

Conclusion

In this study, we conducted by the hydro- distillation, the extraction of essential oil of *Artemisia annua* grown in Cameroon and Luxembourg with an extraction yield of 0.16%. After studies on human volunteers and that conducted in the laboratory, we can conclude that the 2 essential oils could be the alternative to synthetic repellents if properly exploited.

The future scope of this investigation will be to develop an appropriate formulation with suitable synergists, assessment on the product field to determine the effects of toxicology and biological efficiency.

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