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# Repellent properties of essential oils of *Afromomum stipulatum* (Zingiberaceae) from DR Congo against *Anopheles gambiae*

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## ABSTRACT
Essential oils from different plant species were indicated to possess ovicidal, larvicidal and repellent properties against various insect species and are regarded as environmental compatible pesticides. In this study, essential oils of *Afromomum stipulatum* leaves, rhizomes, husk and seeds were evaluated for their repellent effect against malaria vector, *Anopheles gambiae* and their protection effect for human skin. The effective time of the repellent activity increased with concentration of oil. The highest protection time was found to be of one hour. The active compounds could be α-pinene, β-Pinene, β-Caryophyllen, α-humulene and caryophyllene oxide that were found as major compounds of these oils.
Keywords: *Repellent effect, Afromomum stipulatum, essential oils, Anopheles gambiae,*

## INTRODUCTION
Mosquitoes became an important pest in the Democratic Republic of the Congo, where the incidence of infectious diseases is increasing [1,2], and they are vectors of several diseases that affect humans and domestic animals in the world [3]. The deforestation and industrialized farming are two of the factors causing an alarming increase of mosquitoes [4]. The World Health Organization stated that the global warming is also expanding the range of mosquitoes and these are the major vector of several diseases such as malaria, yellow fever, rift valley fever, filariasis, schistosomiasis, Japanese encephalitis, dengue fever [4], putting millions of humans at risk. Due to this change, malaria mosquitoes are found in upland areas where they never been seen before [2]. With the increase of drug resistance, the problem is worsened while attempts are made to control mosquitoes with pesticides but this approach was found to be ineffective [4]. Wearing long pants in wooded areas or disposing of standing water are the measures to minimize the chances of attracting mosquitoes but these measures are not enough. Therefore, the use of mosquito repellent is required in order to fight against mosquitoes meanwhile some studies suggest the use of essential oils to keep mosquitoes away [5,6]. Insect repellents may be chemicals or plant-based products that can prevent from arthropod bites in order to reduce host-vector contact [7]. For many centuries, plants are known to produce secondary metabolites that are found to be physiologically active and have been used for medicinal purposes. It is also known that the repellent activity of aromatic plants is due to essential oils which eventually are used as flavor in food products, odorants in flagrances, pharmaceuticals and as insecticides [8]. The use of repellent to protect humans and animals from bites of mosquitoes has already been accepted as part of an overall integrated mosquito-borne disease control program [9]. A range of phytochemical extracted from various botanical sources were reported to have detrimental effects on mosquitoes [10,11]. Essential oils from different plant species possess ovicidal, larvicidal and repellent properties against various insect species and are regarded as environmental compatible pesticides [12,13]. This study aims to find a way to control mosquito vectors by testing the repellent effect of *A. stipulatum* essential oils collected in the Democratic Republic of the Congo.

## MATERIAL AND METHODS
### Plant Material
For the repellency test, four parts of *A. stipulatum* were used (Table 1). This plant belongs to Zingiberaceae family which constitutes terrestrial rhizomal herbs. Mostly, they are found in tropical areas (Asia and Africa) [14]. The wild specimen was collected around Kahemba and was identified at Herbarium of Sciences Faculty, University of Kinshasa.
### Extraction of Essential Oils
Each sample (350 g of fresh material) of the four parts of plant was submitted to hydrodistillation for 2 hours. The oils obtained by decantation were stored in sealed vials kept in the fridge at -4° C before analysis.
### Repellent bioassay
Tests were carried out on the arms and legs of human volunteers inside cages (30 × 30 × 30 cm) containing mosquitoes according to Beever method in order to determine the repellent effect of essential oils of different parts of the plant [15]. Both arms and legs of human volunteers were carefully covered with thick paper except a 4 $cm^2$ skin portion exposed to mosquito bites.
### Identification of compounds
Some major chemical compounds were identified using GC (AGILENT) and the GC coupled with MS (SHIMADZU) by comparing their mass spectra to those of WILEY 275L database.

## RESULTS AND DISCUSSION
Table 1 give the yield (V/W/%) and oil color of plant parts used for the test.
Table1: Four plant parts used for the test, their yield (V/W/%) and their oil color

| Used parts | Yield | Oil color |
|---|---|---|
| Leaves | 0.17 | Pale yellow |
| Rhizomes | 0.20 | Yellow |

| | | |
|---|---|---|
| Husks | 0.12 | Pale yellow |
| Seeds | 0.30 | Yellow |

The hydrodistillation of these different parts of *A. stipulatum* gave yellowish oil. In fact, Diomandé et *al*. [16] showed that essential oils of species of *Afromomum* are yellow. The highest yield was found in seeds, followed by rhizome, and a slight proportion was obtained from leaves and husks.

Major compounds of essential oil from *A. stipulatum* with their retention time are given n table 2.

Table 2. Major compounds of essential oil from *A. stipulatum* and their retention time

| **Name of compounds** | **RT(min)** |
|---|---|
| α-pinene | 6.375 |
| β-pinene | 6.824 |
| β-caryophyllen | 11.262 |
| α-Humulene | 11.567 |
| caryophillene oxide | 12.664 |

From table 2 above, according to the intensity of chromatographic peaks, it is clearly shown that main components of *A. stipulatum* essential oils are : α-Pinene, β-Pinene, β-Caryophyllen, α-humulene and Caryophyllene oxide. Minor compounds were not determined.

In fact, Ngakegni-Limbili *et al*. [17,18] showed that β-Pinene, 1,8-cineol, α-selinene, β-caryophyllen, are the major compounds of seeds and husks of *A. stipulatum* and *A. giganteum*. Diomandé *et al.* [16] reported that β-pinene was the most abundant compound after analyzing leaves and rhizome oils of *Afromomum* species. The chemical composition of leave oil was dominated by hydrocarbon compounds such as α-pinene, β-pinene, β-caryophyllen, α-humulen and one oxide (caryophillene oxide).

Table 3 shows repellent time and average biting frequency of the essential oil of used parts

Table 3. Repellent Time and average biting frequency of essential oil

| **Repellent time (minutes)** | **Biting Frequency (%)** | | | |
|---|---|---|---|---|
| | **0.0625 ml** | **0.125 ml** | **0.25 ml** | **0.5 ml** |
| 10 | 0 | 0 | 0 | 0 |
| 30 | 0 | 0 | 0 | 0 |
| 60 | 0 | 0 | 0 | 0 |
| 90 | 10 | 5 | 5 | 0 |
| 120 | 85 | 60 | 55 | 25 |
| 150 | 100 | 100 | 85 | 65 |

Table 3 showed that there is a relationship between repellent time and biting frequency. An increase in biting frequency is correlated with an increase in repellent time. While the repellent time increases, the effectiveness of the oil decreases and it was the same for the other parts of the plant.

From low (0.0625 ml) to high (0.5 ml) volume, the biting frequency reappeared after 60 minutes. The estimated time to show the repellent activity against *Anopheles gambiae* was 60 minutes or an hour i.e. the protected effect of essential oils is an hour.

Repellents are to be typically applied to exposed skin or can be applied to clothes or other surfaces in order to discourage the landing of arthropods [7] and Maia and Moore [6], reported that essential oils can be applied each hour on the skin. The effectiveness of the repellent effect decreases while the exposure time increases. Xue *et al* [19] found only 15 minutes of protection with Neem Aura, Sun Swat and Bygone essential oils. Fradin and Day [20] demonstrated that Soybean oil- based repellents provided between 16-195 minutes of protection (average of 1.5 hours). In fact, 1-3 hours may be a good period of exposure to mosquitoes when oil concentration is low [4, 9, 12]. But in areas of intense mosquito activity, higher concentrations may be needed for adequate protection. In addition to *A. stipulatum* oil, another plant oil can be used in order to increase the repellent time. Barnard and Dexue [21] as Fradin and Day [20] reported that repellent time can depend of plant nature.

**Health benefits**

Essential oils are derived from plant parts, and have a specific scent. They are often used in perfumes, cosmetics, room fresheners and flavorings. Their misuse can cause serious poisoning and many people think that essential oils are harmless because they are natural and have been used for long. In some cases, that is simply not true. Some can cause itches on the skin, and other can be poisonous if absorbed through the skin or swallowed [4, 12]. The present study revealed that an essential oil of *A. stipulatum* has a pleasant scent, and non-irritant, non-toxic, not greasy on the skin and harmless to environment. These results confirm those of Petel *et al* [4] and Granett [22] that showed respectively the non-toxicity and pleasant scent of *Afromomum* species essential oils. In fact, the skin is the largest organ of our body and absorbs much of what we apply, thus using less toxic ingredients is the right choice [23].

Individuals can have various reactions to essential oils as it might be to medicines and other products. Essential oils of our studied plant revealed no skin problems in 30 legs and arms tested. Repellent plant materials are intensively used in many villages in Kahemba region and the use of fresh leaves as repellent is popular in this part of the Democratic Republic of the Congo. Fresh leaves of *A. stipulatum* are stored in the house or are hanged up inside the house and within one or two days, the scent released will be spread in the house and move away mosquitoes. Fresh leaves prevent or reduce mosquito attacks or mosquito bites during the night in Congolese villages. Despite that fact that people don't have information on the toxicity of the plant, no environmental or human health problems are known among them. Fresh leaves are assumed to be easily biodegradable, thus their application may greatly be useful than manipulation of synthetic insecticides that pollute the environment.

Our previous study showed that essential oils of *A. stipulatum* can also be used as larvicidal, repellent and imagocidal plant [11]. In fact, Veena *et al* [8], showed that essential oils of *Zingiber officinale* and *Rosmarinus officinale* were found to be ovocidal and repellent.

## CONCLUSION

The present study aimed to show the repellent activity of essential oils from different parts of *A. stipulatum* namely leaves, rhizomes, husks and seeds. The results showed that the essential oils used parts of *A. stipulatum* have repellent effect against *Anopheles gambiae*. This activity would be due to α-pinene, β-Pinene, β-Caryophyllen, and α-humulene and caryophillene oxide. *A. stipulatum* showed no environmental or human health problem. Thus, essential oils of *A. stipulatum* can be used as mosquito repellent.

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