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Research Article

Ovicidal Effect of Essential Oils from Aromatic Plants of Congo-Brazzaville Against *Anopheles gambiae* a Vector Agent of Malaria

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Abstract

Background and Objective: Malaria is a mosquito-caused disease that poses huge problems in the world. The use of synthetic insecticides has several harmful consequences including environmental pollution, poisoning of humans and ineffectiveness due to mosquito resistance. Therefore, the use of essential oils as biological insecticides may be a friendly alternative route. **Materials and Methods:** Eleven aromatic plant materials were used. Essential oils were obtained by hydrodistillation. The study of toxicity against *Anopheles gambiae*'s eggs was carried out after 72 hrs of exposure and the percentage of egg mortality was determined as well as the calculation of LC_{50} . **Results:** The obtained results showed that the highest ovicidal activity was demonstrated by the essential oils of *Chenopodium ambrosioides*, *Cymbopogon citratus* and *Aframomum stipulatum* ($LC_{50} = 0.040 \text{ g L}^{-1}$) followed by *Zingiber officinale* ($LC_{50} = 0.043 \text{ g L}^{-1}$). **Conclusion:** The findings of this research study revealed the toxicity of eleven essential oils against *Anopheles gambiae*'s eggs, a malaria vector agent. Furthermore, these confirm the practice of traditional African medicine and pharmacopoeia. Thus, essential oils can be a source of natural insecticides.

Key words: Malaria, essential oil, ovicidal, eggs, *Anopheles gambiae*, mosquito

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Malaria remains one of the most worrying endemics in black Africa. It is caused by a Hemococcidae of the genus *Plasmodium*, transmitted to humans by a female mosquito of the genus *Anopheles*. Statistically reported data estimated that an approximation of 627000 deaths was related to malaria in 2020, of which 96% occurred in Africa. Additionally, children under 5 years and pregnant women were the most vulnerable segment of the population¹. In the short term, controlling malaria in these countries would make an estimated profit ranging from \$3-12 billion a year. In the Central African Region, Ntonga *et al.*² have published work on the evaluation of the larvicidal activity of three essential oils of plants of Cameroon with an interesting result. Literature reports areas of the world where malaria eradication is being monitored and many other countries from where an intensification of this disease has been observed for several decades^{1,3,4}. This would be linked to an approximate application of prevailing preventive and curative methods, economic problems (high costs of antimalarials drugs, mosquito nets and insecticides) and especially the resistance of plasmodium and the vector, respectively to antimalarials and synthetic insecticides⁵⁻⁷. Also, vector control is an essential component of the fight against malaria and its elimination⁸.

Most vectors are resistant to Dieldrin, DDT, Permethrin, Deltamethrin and Lambda-cyhalothrin^{6,7}. Faced with this phenomenon of resistance, which has become a major obstacle to the prevention and treatment of malaria, the use of natural substances extracted from plants with proven insecticidal properties is more encouraged⁹⁻¹¹. In the African tradition, the utilization of plants as an insecticide has been a very commonly known practice since ancient times and numerous known plant-derived compounds such as pyrethrin, nicotine and rotenone have been used as insect control agents for decades. Moreover, plants have been revealed to be potential sources of new natural insecticides^{9,11}, which is a very important avenue to explore.

The purpose of this study is to evaluate the ovicide activity of the essential oils of eleven aromatic plants of the Congo Brazzaville, for the fight against *Anopheles gambiae*, the vector agent of malaria. This result could be an alternative of synthetic chemicals to natural products better tolerated in the environment.

MATERIALS AND METHODS

Study area: This research project was conducted from 2018 to 2020 in CHIRED-CONGO at the laboratory of medicinal chemistry and pharmacotechnie of medicinal plants, B.P.

13.922 Brazzaville, Republic of the Congo and at the Plant and Life Chemistry Unit, Faculty of Sciences and Techniques, Marien Ngouabi University, Brazzaville B.P. 69, Republic of the Congo.

Plant material: The plant materials named *Cyperus rotundus* (Cyperaceae), *Cyperus esculentus* (Cyperaceae), *Hyptis suaveolens* (Lamiaceae), *Aframomum giganteum* (Zingiberaceae), *Aframomum stipulatum* were collected in green spaces in Brazzaville and the samples: *Cyperus articulatus* (Cyperaceae), *Chenopodium ambrosioides* (Chenopodiaceae), *Guibourtia demeusei* (Fabaceae-Caesalpinioideae), *Cymbopogon citratus* (Poaceae) and *Lippia multiflora* (Verbenaceae) were collected in the Owando town. Plants were identified by Professor Jean-Marie MOUTSAMBOTE and the voucher specimens were registered with the herbarium number as listed below.

Cyperus articulatus Linn J. KOEHLIN No.1396 et J. TROCHAIN No. 9435, *Cyperus rotundus* Linn B. DESCOINGS No. 6649 et J. KOEHLIN No. 489, *Cyperus esculentus* Linn P. SITA No. 38 et J. KOEHLIN No. 1591, *Zingiber officinale* P. SITA No. 378 et J. TROCHAIN No. 1411, *Aframomum giganteum* MJM No. 7661, *Aframomum stipulatum* MJM No. 7662, *Chenopodium ambrosioides* MJM No. 7663, *Lippia multiflora* A. BOUQUET No. 1411 et J. KOEHLIN No. 1443, *Cymbopogon citratus* MJM No. 7659, *Hyptis suaveolens* P. SITA No. 278 et J. TROCHAIN No. 11411, *Guibourtia demeusei* J. TROCHAIN No. 11471 et C. DONIS No. 2379 and deposited at the national herbarium. These fresh plant materials were dried by spreading them on a bench, devoid of sunlight for three weeks in the Plant and Life Chemistry Unit at the Faculty of Sciences and Technologies of the Marien Ngouabi University of Brazzaville-Congo.

Essential oils extraction: Dried plants were powdered using a mechanical grinder and 260 g of dry leaves of *Hyptis suaveolens*, *Chenopodium ambrosioides*, *Cymbopogon citratus*, 500 g of the rhizomes of *Zingiber officinale* and 720 g of *Guibourtia demeusei* exudates were each introduced into a 5 L flask, then subjected to hydrodistillation using Clevenger extractor for 4 hrs. The essential oil collected by decantation at the end of the distillation was dried over anhydrous sodium sulfate and then introduced into hermetically sealed dark glass bottles and then kept in the refrigerator at a temperature of 4°C.

Production of egg *Anopheles gambiae*: The larvae of *Anopheles gambiae* were collected at the edge of the river Tsiémé, in the 5th arrondissement (Ouenzé) of the city of Brazzaville. These larvae were transported to the Faculty of



Fig. 1: Eggs of *Anopheles gambiae*

Sciences and Technologies. They were fed with non-creamy cookies for 4 days, in a cubic cage, side 60 cm, surrounded by a non-impregnated mosquito net on their faces, so that adult mosquitoes from the emergence of the larvae do not escape. Several kinds of cotton soaked in glucose solution were placed in the mosquito cage so that they could feed. After 3 days of feeding the adult mosquitoes with glucose solution, an anaesthetized rat was introduced into the mosquito cage for 48 hrs, so that the fertilized females take their blood meal for the good development of their eggs. After 4 days of the blood meal, mosquito eggs were laid in small plastic tubs placed in the mosquito box.

Ovicidal activity test: Ovicidal bioassay was carried out as previously described by Prajapati *et al.*¹¹ with some modifications. Several 25 *Anopheles gambiae* eggs were introduced into each jar and eggs visible using a binocular magnifier coupled to a camera (Fig. 1) are introduced into a 5 cm diameter beaker containing 80 mL of a solution of each sample at concentrations of 0 (control), 0.0125, 0.025, 0.050, 0.100, 0.200 and 0.400 g L⁻¹. The experiments were repeated three times for each concentration. Counting of dead eggs was carried out after 72 hrs of exposure. The percentage of mortality of eggs was calculated by the formula below previously described by Prajapati *et al.*¹¹.

$$\text{Egg mortality (\%)} = \frac{\text{Number of exposed eggs} - \text{Number of larvae}}{\text{Number of exposed eggs}} \times 100$$

RESULTS AND DISCUSSION

Essential oil extraction: Table 1 shows the colour, the yield of essential oils and the organ used for the hydrodistillation of the indicated plant. It has been shown that leaves of

Lippia multiflora and the rhizomes of *Zingiber officinale* were rich in essential oils, with yields of 1.7%. Tsiba *et al.*¹² and Folashade *et al.*¹³ obtained yields of 1.6 and 1.57% for the extraction of essential oils from the leaves of *Lippia multiflora*. These results were in agreement with ours. On the other end, Bassole *et al.*¹⁴ found a 2.2% extraction yield of essentials from the same plant. As for the extraction of the essential oil from the rhizomes of *Zingiber officinale*, we obtained a 1.7% of yield. Sharma *et al.*¹⁵ obtained a 1.26% of yield, which was lower in comparison with our obtained results. Al-Dhahli *et al.*¹⁶ reported that fresh ginger may contain 1-2% of volatile oil. It is commonly known that the variation in the yields of oils could be due to several parameters.

These yields are followed by those of the leaves of the two *Aframomum*, which are around 1.20%. Bossou *et al.*¹⁷ found a yield of 1.3% from *Aframomum*, it was noted, little difference in yield compared to our work. On the other end, Zollo *et al.*¹⁸ found a yield ranging from 0.070-2.6%. Oumarou *et al.*¹⁹ and Bigoga *et al.*²⁰ described, respectively a yield of 1.8 and 1% instead of 0.72% for our extraction, in the case of the leaves of *Chenopodium ambrosioides*, but Tapondjou *et al.*²¹ found a return of 0.8%, a value somewhat close to ours. The extraction yield of essential oil from *Zingiber officinale* rhizomes is 1.7%, a value significantly higher than what was found by Sasidharan and Menon²² who found 1.5%.

We found a yield of 0.53%, for the extraction of essential oil from the rhizomes of *Cyperus articulatus*, this yield is close to 0.59%, the value found by Héritier *et al.*²³, for this same species. Kasali *et al.*²⁴ found a yield of 0.68% for *Cymbopogon citratus* compared to 0.5% obtained for our work.

The yield of the essential oil of *Cyperus rotundus* has been found at 0.33%. This value is significantly higher than those found by Oladipupo *et al.*²⁵, why extracting essential oils from the rhizomes of *Cyperus rotundus* of 0.16 and 0.20%. Several reasons classically justify this kind of delay: The harvest period, the nature of the soil, the climate and even all the manipulations.

Ovicidal activity: Results of the ovicidal activity of essential oils on *Anopheles gambiae* (Table 2) showed the percentage of mortality of eggs, after 78 hrs of exposure to the different concentrations of essential oils of *Cyperus articulatus*, *Cyperus rotundus*, *Cyperus esculentus*, *Aframomum stipulatum*, *Aframomum giganteum*, *Zingiber officinale*, *Chenopodium ambrosioides*, *Lippia multiflora*, *Cymbopogon citratus*, *Hyptis suaveolens* and *Guibourtia demeusei*.

The curves represented in Fig. 2a-b, showed the mortality rates of *Anopheles gambiae* eggs after 72 hrs of exposure at different concentrations.

These results have shown that all essential oils demonstrated remarkable ovicidal activity with the eggs of *Anopheles gambiae* with several levels of toxicity. All of these oils showed 100% mortality at a concentration of 0.4 g L^{-1} (400 ppm). From the concentration of 0.1 g L^{-1} (100 ppm), essential oils of *C. ambrosioides*, *Lippia multiflora*, *Cyperus articulatus*, *Guibourtia demeusei*, *Aframomum stipulatum*,

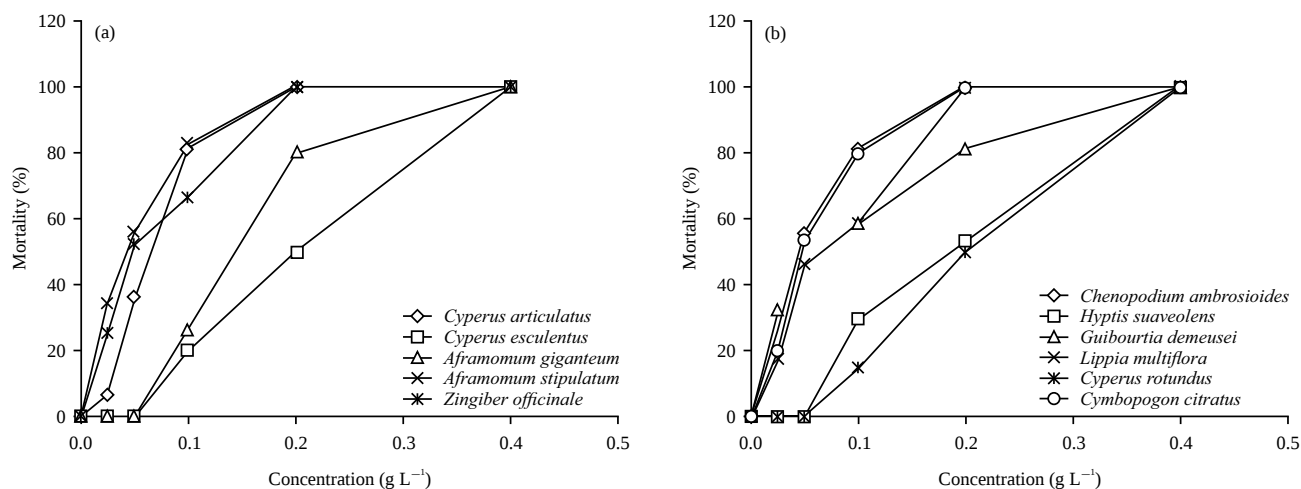
Zingiber officinale and *Cymbopogon citratus* showed 100% of mortality of *Anopheles gambiae* eggs. These differences in toxicity are because each of these essential oils is composed of molecules with varying degrees of ovicidal effect. These oils prevented the hatching of *Anopheles gambiae* eggs during the 72 hrs of exposure. However, the LC_{50} values obtained from this ovicidal activity showed that the essential oil of *C. ambrosioides*, *C. citratus* and *Aframomum stipulatum* are more toxic to *A. gambiae* eggs with an LC_{50} of 40 ppm followed by the essential oils of *Zingiber officinale* 0.043 g L^{-1}

Table 1: Yields and colours of essentials oils

Plant species	Organs used	Color of HE	Yields (%)
<i>Cyperus articulatus</i>	Rhizomes	Yellow	0.53
<i>Cyperus rotundus</i>	Rhizomes	Pale yellow	0.33
<i>Cyperus esculentus</i>	Rhizomes	Pale yellow	0.12
<i>Aframomum stipulatum</i>	Leaves	Yellow	1.20
<i>Aframomum giganteum</i>	Leaves	Yellow	1.20
<i>Zingiber officinale</i>	Rhizomes	Colorless	1.70
<i>Chenopodium ambrosioides</i>	Leaves	Dark yellow	0.72
<i>Lippia multiflora</i>	Leaves	Yellow	1.70
<i>Cymbopogon citratus</i>	Leaves	Yellow	0.50
<i>Hyptis suaveolens</i>	Leaves	Yellow	0.05
<i>Guibourtia demeusei</i>	Exudates	Colorless	0.21

Table 2: Mortality of eggs exposed to increasing concentrations of essential oils in percent and LC_{50}

Concentration samples	0.4 g L^{-1}	0.2 g L^{-1}	0.1 g L^{-1}	0.05 g L^{-1}	0.025 g L^{-1}	0 g L^{-1}	LC_{50}
<i>Chenopodium ambrosioides</i>	100	100.00 ± 0.00	81.33 ± 2.67	56.00 ± 0.00	26.68 ± 2.21	0	0.040
<i>Hyptis suaveolens</i>	100	53.32 ± 1.77	29.32 ± 1.77	00.00 ± 0.00	00.00 ± 0.00	0	0.150
<i>Guibourtia demeusei</i>	100	100.00 ± 0.00	81.33 ± 1.67	58.67 ± 1.77	32.00 ± 0.00	0	0.075
<i>Lippia multiflora</i>	100	100.00 ± 0.00	58.68 ± 1.77	46.00 ± 0.00	17.32 ± 1.77	0	0.060
<i>Cyperus articulatus</i>	100	100.00 ± 0.00	81.65 ± 0.33	36.33 ± 2.67	6.67 ± 0.88	0	0.075
<i>Cyperus rotundus</i>	100	50.00 ± 1.77	14.68 ± 0.00	00.00 ± 0.00	00.00 ± 0.00	0	0.200
<i>Cyperus esculentus</i>	100	50.06 ± 1.77	20.00 ± 0.00	00.00 ± 0.00	00.00 ± 0.00	0	0.200
<i>Aframomum giganteum</i>	100	80.00 ± 0.00	26.67 ± 1.77	00.00 ± 0.00	00.00 ± 0.00	0	0.140
<i>Aframomum stipulatum</i>	100	100.00 ± 0.00	82.67 ± 2.22	56.00 ± 0.00	34.67 ± 2.67	0	0.040
<i>Zingiber officinale</i>	100	100.00 ± 0.00	66.67 ± 2.67	52.00 ± 0.00	25.32 ± 1.67	0	0.043
<i>Cymbopogon citratus</i>	100	100.00 ± 0.00	80.00 ± 0.00	53.32 ± 1.77	20.00 ± 0.00	0	0.040

Fig. 2(a-b): Evolution of *Anopheles gambiae* egg mortality after 72 hrs of exposure to different concentrations of essential oils

($LC_{50} = 43$ ppm), *Lippia multiflora* ($LC_{50} = 60$ ppm), *Cyperus articulatus* ($LC_{50} = 75$ ppm), *Guibourtia demeusei* ($LC_{50} = 0.75$ g L^{-1}), the other oils are less toxic. Based on the difference in the LC_{50} values, there are therefore clearly a lot of considerable and significant differences between these essential oils.

CONCLUSION

The finding of this research study gave us a clear indication of the toxicity of essential oils in the fight against *Anopheles gambiae*, a malaria vector agent. Additionally, these tested samples showed remarkable mortality rates from eggs *Anopheles*. These research results encouraged the use of biodegradable essential oils as insecticides in the control of vectors against malaria and could contribute to the study of Congolese medicinal plants in these aspects relating to the recognition of traditional African medicine and pharmacopoeia.

SIGNIFICANCE STATEMENT

This study is the first to study the effect on *Anopheles* eggs by our team. The present work confirmed that eggs should be more sensitive to essential oil than mosquitoes and larvae as many authors describe it for several other insects. The practice of the ovicidal concept may give more benefits. First, the use of small amounts of the active substance will lead to an economic benefit and then will reduce adverse environmental effects. Further investigation will be carried out on more active essential oils, which is a source of new natural product that should be used with less hazardous to human health and the environment.

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