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Contribution to vector control by essential oils of *Aucoumea klaineana, Canarium schweinfurthii, Cymbopogon nardus, Dacryodes edulis and Eucalyptus citriodora* from Gabon

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ABSTRACT

Introduction: *Aucoumea klaineana*, *Canarium schweinfurthii*, *Cymbopogon nardus*, *Dacryodes edulis,* and *Eucalyptus citriodora* are of Gabonese origin, believed to have insecticidal activity. This study contributes to vector control by the insecticidal activities (larvicidal and ovicidal) of five essential oils from these plants against *Anopheles gambiae*, a major vector of malaria in Gabon.
Methods: The essential oils were extracted by hydrodistillation. Larvicidal and ovicidal effects of essential oils were performed using different concentrations on third and fourth instar larvae and eggs of *A. gambiae* vectors. The effects of these oils were examined on the mortality rate of larvae and eggs.
Results: The essential oils of *A. klaineana*, *C. schweinfurthii*, *C. nardus*, *A. edulis,* and *E. citriodora* showed greater activity against mosquito larvae ($7.33 < LC_{50} < 107.14$) compared to eggs ($22.80 < LC_{50} < 64.63$). *D. edulis* showed the highest activity against *Anopheles gambiae* eggs and larvae. Of all plants, essential oils from *A. klaineana* showed the lowest activity of *A. gambiae* eggs and larvae. Larvae were more sensitive than eggs. All essential oils were toxic to the various aquatic vectors of malaria.
Conclusion: The study reveals the potential ovicidal efficacy and larvicidal activity of these plants against *A. gambiae*.

Implication for health policy/practice/research/medical education:
Essential oils from *Aucoumea klaineana, Canarium schweinfurthii, Cymbopogon nardus, Dacryodes edulis,* and *Eucalyptus citriodora* have shown effects against mosquito larvae and eggs. These aromatic plants are good candidates for vector control.
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Introduction

Gabon has plant biodiversity rich in natural products, particularly aromatic and medicinal plants (1,2). Essential oils (volatile compounds) have many medicinal properties and act in several areas of health (aromatherapy). Several aromatic plants in Gabon are used against mosquitoes (3-5). Malaria continues to dominate the public health spectrum, especially on the African continent, where the *Anopheles gambiae* complex is present in endemic regions. This species complex is the vector of *Plasmodium*. Malaria kills between 1.4 and 2.7 million of the estimated 300 to 500 million clinical cases each year (6). An alternative approach to mosquito control is the use of natural, plant-derived products. The vast majority of essential oils have pleasant smells and constitute elements of defense against the attack of parasites, herbivorous animals and insects (4,5). *Aucoumea klaineana* is the main tree species exploited in Gabon (7). Numerous scientific

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studies have demonstrated the antioxidant, insecticidal, and antimicrobial activities of the volatile compounds extracted from its resin. Studies conducted on the essential oil of *Dacryodes edulis* have revealed that its resin could be used as varnish and incense. Bark powder is used to treat wounds of non-traumatic origin or ulcerative wounds (8). This plant is also used to treat cases of dysentery and fevers (9). The very fragrant bark of *Canarium schweinfurthii* exudes a fragrant resin, which becomes opaque in the air. Studies conducted on *C. schweinfurthii* oleoresin revealed the antioxidant, antiradical, anti-inflammatory, and antimicrobial activities of the derived volatile compounds (3,10). *Cymbopogon nardus* is an herbaceous plant found in different regions of Africa and the West Indies (11,12). In Central Africa, citronella is traditionally planted around houses because its smell is said to repel mosquitoes (13). *Eucalyptus citriodora* is a large evergreen tree native to Australia and Tasmania. It is widely cultivated in many other countries, including Congo Basin countries. Its essential oil is used in many processes for formulating perfumes and disinfectants. Citronellal is used for the synthesis of menthol and citronellol. The leaves have antiseptic properties and are used in the treatment of many skin diseases. *E. citriodora* essential oil has antibacterial, antifungal, acaricidal, and insect-repellent activities. This essential oil is phytotoxic and herbicidal activity. In traditional medicine, this essential oil is used as an antispasmodic agent (14). This study was designed specifically to investigate the effects of essential oils of *A. klaineana*, *C. schweinfurthii*, *C. nardus*, *D. edulis,* and *E. citriodora* on eggs and larvae of the *Anopheles gambiae* complex, vector of *Plasmodium* in Gabon.

Materials and Methods

Plant materials

The plants were authenticated by a botanist at the National Herbarium of the Institute of Pharmacopoeia and Traditional Medicine (IPHAMETRA) in Libreville, and their specimens were deposited there. The resin of *A. klaineana* (voucher number: KA01) and *D. edulis* (voucher number: DE 02) were harvested in July 2021, in Mounana. *C. schweinfurthii* (voucher number: CS 03) resin, which turned slightly yellow when dried, was harvested in June 2021 in the village of Mebane Endama Minkong, 23 km from Oyem. The leaves of *E. citriodora* (voucher number: EC 04) and *C. nardus* (voucher number: CN 05) were made in Franceville in September 2021. To harvest the resin, the scarifications on the trunks of the species were carried out in order to let it exude. After a week, considerable quantities of this resin could be collected. The aerial parts of the leaves of *E. citriodora* and *C. nardus* were harvested and dried for 72 hours under an air-conditioned shelter before extraction.

Extraction

The essential oils were extracted by hydro-distillation method using Clevenger-type equipment. The extraction was carried out at 40°C for 4 hours for each plant depending on whether the production of the essential oil by the part of the chosen plant was abundant or not. The essential oils obtained were stored in the refrigerator at 4°C. The extraction yields (R) were calculated using the ratio of the mass of the essential oil to the plant mass (leaves or resins) by the following equation:
$R\ (\%) = (W_{EO}/W) \times 100$.

Where W_{EO} is the mass of essential oil (g), and W is the weight of dry leaves or resins (g).

Mosquito eggs and larvae

Anopheles gambiae larvae and eggs were collected in various sites in Franceville Gabon and reared to adults in the insectary that were used in the bioassays. Members of the *A. gambiae* complex were identified from a previous collection in Goden.

Larvicidal effect bioassays

Essential oils of the plants were tested according to the standard WHO protocol (15), with slight modifications (16,17). For the experimental treatment, 1 mL of test solution was mixed with 224 mL of distilled water in a plastic cup. Then, twenty 3rd and 4th instar larvae, gathered in 25 mL of distilled water, were transferred to the cup. Each replicate set included two *A. gambiae* batched for each dose tested and one control, consisted of 1 mL of absolute ethanol in 249 mL of distilled water**.** After a period of 24 hours, mortality counts were performed. Dead larvae were identified when they did not arouse after probing with a needle on the siphon or the cervical region. Moribund larvae were those unable to rise to the surface (within a reasonable period of time) or unable to show the characteristic diving reaction when the water was disturbed. They also showed discoloration, unnatural positions, tremors, uncoordinated, or rigor. After each replicate, moribund and dead larvae were combined and expressed as percent mortality at each concentration. Each test spanned 3 or 4 replicates and the replicates with ≥15% mortality in the control group were discarded from the analysis.

Ovicidal efficacy bioassays

One mL of test solution was mixed with 29 mL of distilled water in six plastic cups (115 mm diameter and 80 mm depth). Then, 20 recently-laid eggs were held in the insectary, and the egg mortality was scored 24 hours post-treatment. Eggs that did not hatch after the incubation period (24 hours) were considered as dead. A total of three replicates were carried out.

Study of acute general ovicidal and larvicidal activity

In a series of 5 batches, containing equal volumes of 10 mL of distilled water, each batch received a given dose of variable essential oil concentrations per batch (100 µL, 80 µL, 50 µL, 30 µL, and 10 µL). Ten larvae or eggs were added to each batch (including a control batch containing only distilled water and larvae/eggs). Each batch was identified by a different number and mark of essential oil concentration. The larvae or eggs were left to incubate for 30 min in each concentration, and then the number of dead larvae or eggs was identified. The 50% lethal concentration (LC_{50}) calculation method was that described by Miller and Tainter (16). It consisted of writing directly on Log probit, the mortality rate as a function of the log of the essential oil concentration.

Statistical analysis

Data were expressed as mean ± SEM. A one-way analysis of variance was used to analyze the data. P <0.01 represented a significant difference between means (Duncan's multiple range test). The toxicity (LC_{50}) of each essential oil was obtained using the regression line given by Excel software. Log probit was the death rate as a function of the log of the essential oil concentration.

Results

Extraction yield

Air-dried leaves and resin of the plants were subjected to hydro-distillation using a Clevenger-type apparatus, and the oils were obtained in the yield of 10%, 6.86%, 3.62%, 1.36%, and 0.60% for *D. edulis, C. schweinfurthii, A. klaineana, E. citriodora,* and *C. nardus*, respectively (Figure 1).

Results of the ovicidal and larvicidal activity of essential oils after 30 minutes

The toxicity (LC_{50}) of essential oils was obtained using the regression line given by the Excel software. We considered the criteria of Komalamisra and Bucker for larvicidal and ovicidal activities, according to which essential oils show not toxic when CL_{50} >750 µg/mL; lower activity when CL_{50} between 200 µg/mL and 750 µg/mL; moderate when CL_{50} between 100 µg/mL and 200 µg/mL; efficacy when CL_{50} between 50 µg/mL and 100 µg/mL and very efficacy when CL_{50} < 50 µg/mL (Table 1).

Essential oils showed greater activity against mosquito larvae (7.33 <LC_{50} <107.14) than against their eggs (22.80 <LC_{50} <64.63) (Table 1).

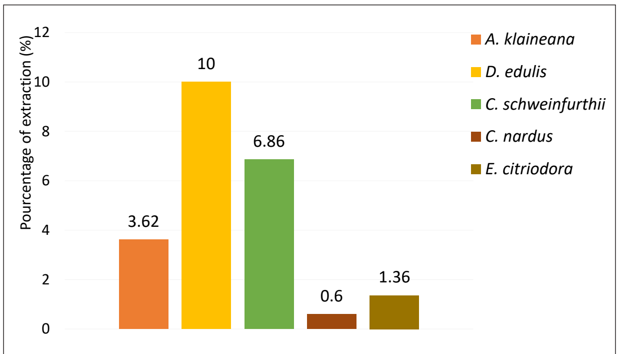

Figure 1. Extraction yield.

Kinetics of mortality of mosquito eggs and larvae

Kinetics of mosquito egg mortality

The kinetic study of the mortality of mosquito eggs, as a function of the concentration of each essential oil, was carried out after 30 min (Figure 2).

The mortality rate from *C. schweinfurthii* essential oil began to increase when its concentration was around 40 µg/mL. This late growth testified to a slow activity of this essential oil compared to the four other essential oils, which on the other hand, showed high ovicidal activities at low concentrations. The essential oil of *A. klaineana* was the only one whose mortality rate on mosquito eggs did not reach 100% after 30 minutes.

Kinetics of mosquito larvae mortality

After 30 minutes, the kinetic study of the mortality of mosquito larvae as a function of the concentration of each essential oil gave remarkable results (Figure 3). Indeed, about 50% of the larvae mortality was noted for four of the five essential oils at concentrations around 20 µg/mL. The essential oil of *C. nardus* was the only one that could not reach 100% of the larval mortality rate after 30 minutes.

Discussion

The best yield (10%) was obtained by *D. edulis* for the resins, and *E. citriodora* had the best extraction yield (1.36%) for the leaves. Resins are more abundant in essential oils than leaves. The resin essential oil of *A. klaineana* was slightly yellowish; this coloring is a function of the degree of oxidation of the resin, a clear essential oil with fresh resin. The extraction yield of *A. klaineana* (3.62%) was relatively close to those reported in the scientific bibliography: 3.3% (2-5) and 5% (7,17,18). However, variations have been observed with different extraction yields. These variations in yields can have several causes. The extraction technique, the stage of maturity of the plant, the place, and the harvesting period of the resin. The type of the solvent used and the genetic factors can also influence the extraction yield of an essential oil (14). The essential oil of *C. schweinfurthii* resin had a yellowish color. The quantity of essential oil obtained from the resin of *C. schweinfurthii* (6.86%) was close to that given by the scientific bibliography: 5.3%, 7.2 (19), and 9.17% (3,4,20).

The resin of *D. edulis* gave a clear essential oil with an extraction yield of 10% (Figure 1); this result was better compared to that obtained by Obame-Engonga et al (21), i.e. 0.68%. The geographic difference in the places, where the resins were harvested could be the source of yield variations (21). They, harvested their resin at Libreville (North-West of Gabon), whereas, for the present study, the resin was collected in Franceville (South-East of Gabon). The geographical difference could influence the intrinsic characteristics of the resin, such as its quantity and/or its

Table 1. Ovicidal and larvicidal activities against *Anopheles gambiae*

Essential oils	Regression equation	R^2	LC_{50} (µg/mL)	Activity
		Ovicidal activity		
A. klaineana	Y= 0.6215X + 8.6577	0.9621	60.63	Efficacy
C. schweinfurthii	Y=1.4530X – 48.110	0.9996	64.67	Efficacy
D. edulis	Y=1.9850X + 7.0091	0.9952	22.80	Very efficacy
E. citriodora	Y= 0.9543X + 14. 206	0.9317	37.88	Very efficacy
C. nardus	Y = 1.1388X + 5.3367	0.9688	37.10	Very efficacy
		Larvicidal activity		
A. klaineana	Y= 1.8263X + 7.7912	0.9845	23.16	Very efficacy
D. edulis	Y= 3.8180X + 11.9042	0.9980	9.49	Very efficacy
C. schweinfurthii	Y=3.7521X + 11.9540	0.9703	10.41	Very efficacy
E. citriodora	Y = 6.8186X + 0.0630	1	7.63	Very efficacy
C. nardus	Y=7491X – 30.737	0.9932	107.14	Moderate

LC_{50}, 50% lethal concentration.

quality in essential oil. Since essential oils are volatile, poor conditioning of this resin or too long a conditioning time could also be the cause of the small amount of essential oil obtained by Obame et al. Manika et al (14) have also shown that a plant can have different extraction yields in the same year, depending on the month when the harvest is carried out. This is a probable cause of the drop in yield obtained by Obame et al compared to that of this study. The percentage of extraction obtained from the essential oil of *C. nardus* (0.6%) is close to 0.67 ± 0.07% obtained in *C. citratus,* which is a plant of the same genus (22). These results may suggest that plants of this genus give low extraction yields of essential oils. The extraction rate of the essential oil contained in the leaves of *E. citriodora* was 1.36%. This rate is included in the usual margin provided by this plant species, ie between 1 and 2.1% (14).

The essential oil of *C. nardus* was clear with a characteristic odor of lemongrass, while *E. citriodora* essential oil was slightly yellow with a lemon odor. The essential oil of *A. klaineana* was yellowish in color, *D. edulis* essential oil was clear, and the essential oil of *C. schweinfurthii* turned slightly yellow.

All essential oils are toxic to the various aquatic vectors of malaria. Such a result could be due to the synergistic effects between the different constituents of these essential oils (23). Also, the majority presence of monoterpenes highlighted by certain authors in these different essential oils could justify the results obtained (2,12,14,20-22). Indeed, the high toxicity of monoterpenes on insect larvae has been demonstrated by Santos (24). On the other hand, compounds such as citral (11), citronellal, citronellol, geranial, and limonene are used in cosmetics for their fragrant, insect repellant, and insecticide properties (25). Studies have been reported on the presence of these compounds in different essential oils (2,12,14,20-22).

The high mortality rates of each of the essential oils

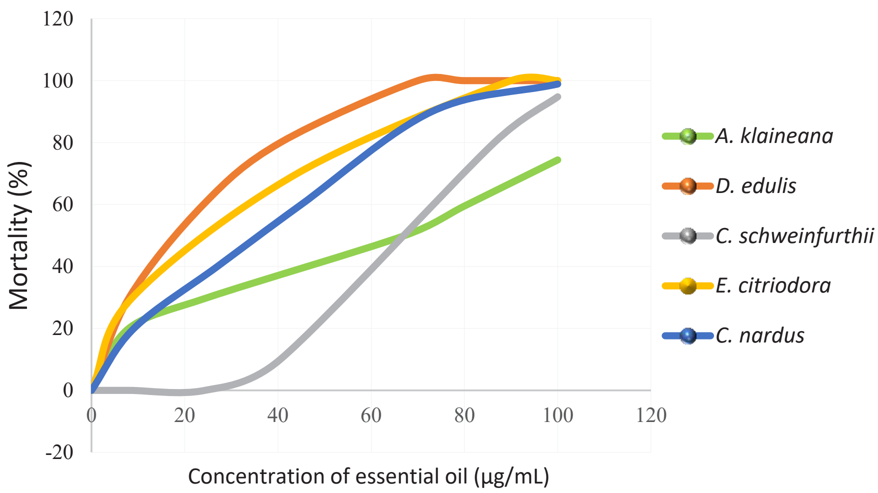

Figure 2. Mortality rate of mosquito eggs.

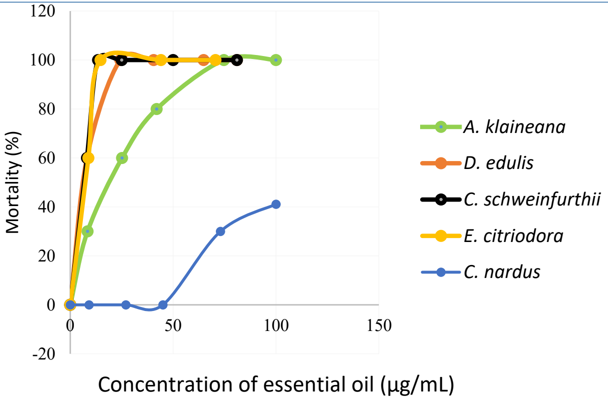

Figure 3. Mortality rate of mosquito larvae.

could justify their uses in African traditional medicine as an insect repellent. This is the case for the essential oil of *E. citriodora* (14), as a mosquito repellent and for the essential oil of *C. nardus* as an insecticide (11). The larvicidal and ovicidal activities of the essential oils of *A. klaineana, D. edulis,* and *C. schweinfurthii* were carried out by Obame, but the insecticidal and repellant activities of the essential oils of *A. klaineana*, *D. edulis,* and *C. schweinfurthii* are not mentioned in the scientific bibliography (4). This study reveals the effectiveness of the actions of these different essential oils in these areas.

A recent study reported that the activity of *C. nardus* essential oil decreased over time (11,13). Thus, it is quite possible that after a certain time the active ingredients present in this essential oil have volatilized. Another study recommended for the vector control of malaria to set up semi-solid formulations of essential oils in place of strictly liquid formulations, which are too volatile and, therefore, quickly ineffective beyond a few hours (26,27).

Conclusion

This study highlights the ovicidal and larvicidal activities of the essential oils of *A. klaineana, C. schweinfurthii, C. nardus, D. edulis,* and *E. citriodora.* Essential oils have greater activity against mosquito larvae. Overall, the different essential oils have been found to be all toxic against the aquatic vectors of malaria. The high mortality rates of each essential oil could justify their use in African traditional medicine as insect repellent, mosquito repellant, and insecticide. Thus, some essential oils have been shown to have more activity than others tested. Also, faced with the many problems of toxicities caused by the use of insecticides of chemical origins, the scientific community turns to the use of insecticides of biological origins towards the use of essential oils, which have low toxicity to the environment.

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Authors' contributions

SOC performed the tests, analyzed the results, and wrote the manuscript. NMT helped with extractions and financing. NMMRL and OBJO contributed to the realization of the ovicidal and larvicidal tests. OELC supervised and corrected the study. All authors read and confirm the final version of the document for publication.

Conflict of interests

The authors declare there is no conflict of interest.

Ethics considerations

The study protocol was approved by the Ethics Committee of Gabon (N °005 march 2011).

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