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Larvicidal and ovicidal properties against *Anopheles gambiae*, antioxidant and antibacterial activities of the combination of essential oils *Eucalyptus citriodora, Cymbopogon giganteus* and *Cymbopogon nardus* from Gabon

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***Abstract*— Plants have received increased attention from scientists as they serve as a rich source for novel natural substances possessing insecticidal properties which are safe to human and ecosystem. As part of ongoing research on the ovicidal and larvicidal proprieties against *Anopheles gambiae*, antioxidant and antimicrobial activities of the combination of essential oils extracted from *Eucalyptus citriodora, Cymbopogon giganteus* and *Cymbopogon nardus* medicinal plants of Gabon. Antioxidant Activity Index (AAI) was determined for antioxidant property evaluation by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. The essential oils were tested against 11 bacteria by using disc diffusion and microdilution methods. The ovicidal and larvicidal activities were evalued by WHO, standard protocol against 3rd and 4th instar larvae of field-collected mosquitoes vectors of human disease members of *Anopheles gambiae.* In this study, the antioxidant activities are poor, moderate and strong for *C. nardus*, *C. giganteus* and *E. citriodora*, respectively. Paired combinations of *E. citriodora*, *C. giganteus* and *C. nardus* had very strong effects (AAI values ranged from 11.57 and 20.46) in comparison to the activities of vitamin C and BHT. All combinations showed an antimicrobial activity stronger than essential oils alone, Ampicillin, Gentamicin and Tetracycline against microorganisms tested. The interaction antagonism or addition, indifferent and synergy, between two oils, depend on the concentrations of the single component and the overall susceptibility of the target microorganism. All combinations showed synergistic effects against *Echerichia coli* 105182 CIP, *Enterobacter aeogenes* CIP 104725, *Enterococcus faecalis* 103907 CIP and *Listeria monocytogenes* CRBIP 13134. An additive effect was observed for *Streptococcus pyogenes*. Combinations showed very efficacy action and lethal activity against *Anopheles gambiae* eggs and larvae. Combinations of *E. citriodora* with *C. nardus* or *C. giganteus* exhibited the best larvicidal action (7.33 µg/mL) and ovicidal activity (13.64 µg/mL), respectively. Eggs were more susceptible than larvae with combinations of *C. giganteus* with *C. nardus* or *E. citriodora*. The use of natural products of plant origin constitutes an alternative approach for malaria control and better efficacy for combating various infections and dry resistance. The result may be useful for the combinations of *Eucalyptus citriodora, Cymbopogon giganteus* and *Cymbopogon nardus* essential oils against pathogen bacteria and *Anopheles gambiae* eggs or larvae.**

Keywords*— Ovicidal and larvicidal proprieties, Anopheles gambiae, antioxidant and antimicrobial activities, combination of essential oils.

I. INTRODUCTION

Malaria attacks about 300 to 500 million people each year, mostly in the tropics, and causes 3 million deaths annually, including 1 child every 30 sec (1). About 90% of this burden is recorded in Africa. Almost half of the world population is at risk from this disease (2). Malaria is caused by intracellular protozoan parasite of genus *Plasmodium* (3). *P. falciparum, P. vivax, P. malariae* and *P. ovale* are four species responsible for human malaria. *P. falciparum* is the most serious (4). Mosquitoes are the major vectors for the transmission of malaria. During the last decade, various studies on natural plant products against mosquito vectors indicate them as possible alternatives to synthetic and chemical insecticides. Natural products from plants are alternative sources of insect control agents since they contain a range of bioactive chemicals, which are selective and do not harm non-target organisms and the environment (5;6). Plants have formed the basis of natural pesticides that make excellent leads for new pesticide development (7). The vast majority of essential oils are pleasant smell and its metabolic and evolutionary significance lies in the role they play as attractor of pollinating agents for its pleasant aroma, constitute elements of defense against the attack of parasites, herbivorous animals and insects. An alternative approach for mosquito control is the use of natural product of plant origin. The plant is traditionally used in treatment of malaria and defense against mosquitoes (8). Plant has been an increased interest in the use of natural antimicrobial agents thus the use of these combinations is strategies to control food-borne bacteria and other pathogenic microorganisms (5;6;7;9). In Central Africa, *Cymbopogon* is traditionally planted in the vicinity of houses because its smell would repel mosquitoes. The most common problem associated with the repellent and insecticidal effects of lemongrass essential oil is that its effectiveness does not exceed a few hours. Lemongrass, on the other hand, has the benefit of being considerably less toxic to humans than other commercial insecticides. The duration of the repellent effect of lemongrass can be moderately increased by favoring semi-solid (gelatinous) topical compositions instead of strictly liquid formulations which are too volatile and therefore rapidly ineffective beyond a few hours (10). In the context of the fight against malaria in sub-Saharan countries, studies have shown that certain *Anopheles gambiae* mosquitoes that have become resistant to commercial insecticides based on derivatives of various natural and synthetic pyrethroids could see their proliferation effectively fought through to essential oil components of certain plants including lemongrass and other members of the genus *Cymbopogon* (Poaceae). The chemical composition of *Cymbopogon citratus* essential oil predominantly contains monoterpenoids: geranial (32.82%), neral (30.21%), myrcene (10.4%) and geraniol (8.19%) (11). The components of *C. nardus* essential oil have proved to be much more repellent, even mutagenic, in synergy with citral to parasitic Leishmania, than pure citral. This essential oil has also been proposed to keep away the anthrax flies that bite domestic animals. Citral and other monoterpenes in this essential oil demonstrated repellent and insecticidal activities for domestic flies and allowed the production of commercial insecticides with almost zero toxicity for humans (12). For *Cymbopogon giganteus* essential oil, the major constituents found are: trans-paramentha- 1(7), 8-dien-2-ol (31.9%), trans-para-mentha-2, 8-dien-1-ol (19.6%), cis-paramentha- 2,8-dien-1-ol (7.2%) and trans-piperitol (6.3%). Its composition out of limonene (6.3%) approaches that (7.7%) found by Alitonou et *al*. (13). Nyamador et *al*. (2010) showed that the leaves of *Cymbopogon giganteus* collected in Togo mainly made up of limonene (23%) followed of para-mentha-2,8- dien-1-ol duplicated between trans forms (5.63%) and cis forms (14.3%).

Eucalyptus citriodora (Poaceae) is a large evergreen tree, native to Australia and Tasmania and successfully introduced worldwide, now widely cultivated in many other countries including Congo Basin countries. *E. citriodora* grows naturally on undulating plateaus, including dry crests, in open and wooded forests, on generally poor soils, 80 to 800 m above sea level. The species can survive a severe dry season (14). Manika et *al*. (15), shows that the chemical composition of the essential oil of *E. citriodora* predominantly contains citronellal (72.6%); citronellol (8.7%); linalool (5.1%) and isopulegol (2.5%). This essential oil is widely used in many fragrance and disinfectant formulation processes. The citronellal obtained from the essential oil is mainly used for the synthesis of menthol and citronellol. The sheets according to the use process have antiseptic properties and are used in the treatment of many skin diseases. The essential oil of *Eucalyptus citriodora* possesses antibacterial, antifungal, ascaricidal and insect repellent activities. This essential oil is potentially phytotoxic and is used as a herbicide. In traditional medicine, this essential oil is used as an antispasmodic (15).

In the present work, we report the ovicidal and larvicidal activities against *Anopheles gambiae*, antioxidant and antimicrobial proprieties of essential oils in combination, extracted from *Eucalyptus citriodora, Cymbopogon giganteus* and *Cymbopogon nardus* medicinal plants of Gabon with the aim to contributing to the search for beneficial uses of these plants. One solution is the application of essential oils or their components in combination.

II. MATERIALS AND METHODS

A. Plant material

The leaves of *Eucalyptus citriodora, Cymbopogon giganteus* and *Cymbopogon nardus* were collected in August 2016 from Mbaya village, Franceville-Gabon. Plants were identified at Herbarium IPHAMETRA. A voucher specimens have been deposited under numbers 73, 74 and 75 for *Eucalyptus citriodora, Cymbopogon giganteus* and *Cymbopogon nardus* respectively at Laboratory of Research in

Biochemistry (LAREBIO), University of Sciences and Technology of Masuku, Franceville, Gabon.
The leaves were hydro distilled for 3 h using a clevenger-type apparatus. The essential oils were dried after decantation, over anhydrous sodium sulfate, filtered and stored in a scaled vial in the dark at 4°C before analysis. The essential oil yield was calculated on the basis of report/ratio (mass of dry oil extracted to the mass of the leaves).

B. Microbial strains

The essential oils of leaves from *Eucalyptus citriodora, Cymbopogon giganteus* and *Cymbopogon nardus* were tested against a panel of resistant microorganisms. The bacteria used were: *Bacillus cereus* LMG 13569 BHI, *Escherichia coli* CIP 105182, *Enterobacter aerogenes* CIP 104725, *Enterococcus faecalis* CIP 103907, *Listeria monocytogenes* CRBIP 13.134, *Pseudomonas aeruginosa* CRBIP 19.249, *Salmonella enterica* CIP 105150, *Salmonella typhimurium* ATCC 13311, *Shigella dysenteriae* CIP 5451, *Staphylococcus aureus* ATCC 9144 and *Streptococcus pyogenes*.

C. Mosquito eggs and larvae

Anopheles gambiae larvae and eggs were collected in various sitting in Franceville; Gabon reared to adults in the insectary was used in the bioassays. Members of *Anopheles gambiae* complex are not identified but from previous collection in Goden.

D. Antioxidant Activity Index (AAI)

The antioxidant activity index was assessed according to the method described by Scherer and Godoy (16). This method is based on DPPH radical test. Briefly, the working reagent was prepared by dissolving 10 mg of DPPH in 100 mL ethanol. Graded concentrations of essential oils ranging from 0.781 to 100 µg/mL obtained by two-fold dilutions were prepared and 100 µL of each dilution were mixed with 100 µL of the working solution of DPPH in a 96-well plate. Absorbancies were measured at 517 nm after 15 min incubation at room temperature in the dark. Vitamin C and butylated hydroxytoluene (BHT) were used as references. The ability to scavenge DPPH radical was calculated by the following equation:
% RSA= 100 x [(A control – A sample)]/ A control. A = Absorbance at 517 nm. The IC_{50} (concentration providing 50% inhibition) of extracts and standards was determinate using regression curves in the linear range of concentrations. The AAI was then calculated as follows:
AAI= ([DPPH] (µg/mL)) /IC_{50} (µg/mL), [DPPH] is the final concentration of DPPH. We considered criteria of Scherer and Godoy (16) according to which plant extracts show poor antioxidant activity when AAI < 0.5, moderate antioxidant activity when AAI between 0.5 and 1.0, strong antioxidant activity when AAI between 1.0 and 2.0, and very strong when AAI > 2.0.

E. Antimicrobial assay

Disk diffusion assay: The agar disk diffusion method was employed for the screening of antimicrobial activities of the essential oils (17;18;19). The test was performed in sterile Petri dishes (90 mm diameter) containing solid and sterile Mueller–Hinton agar medium (Becton, Dickinson, USA). The essential oils absorbed on sterile paper discs (5 µL per Whatman disc of 6 mm diameter), were placed on the surface of the media previously inoculated with 100 µL of overnight microbial suspension (10^8 CFU/mL). One filter paper disc was placed per Petri dish in order to avoid a possible additive activity. Every dish was sealed with laboratory film to avoid evaporation and then incubated aerobically at either 30°C or 37°C according to bacteria for 18–24 h, followed by measurement of the zone diameter of the inhibition expressed in mm. Antibiotic discs of Ampicillin (Am. = 30 µg), Gentamicin (Gen. = 10 µg) and Tetracycline (Te. = 30 µg) were used as positive controls.

Determination of minimal inhibitory concentration (MIC): The minimal inhibition concentration (MIC) values were studied for the bacterial strains which were sensitive to the essential oil in disc diffusion assay. Minimal inhibition concentration (MIC) values were determined using micro-well dilution assay method (19; 20; 21). A serial doubling two-fold dilution of either essential oil was prepared in a microtiter tray over the range 0.075–20 mg/mL in 100 µL Mueller–Hinton broth. The broth was supplemented with ethanol absolute at a concentration of 0.5% in order to enhance essential oils solubility. Overnight broth cultures of each strain were prepared from 18 h broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. An aliquot 100 µL of the inoculum was added to diluted essential oil. The final volume in each well was 200 µL. The plate was covered with a sterile plate sealer. Positive and negative growth controls were included in every test. The tray was incubated aerobically at either 30◦C or 37◦C according to bacteria for 18–24 h. The MIC is defined as the lowest concentration of the essential oil at which the microorganism tested does not demonstrate visible growth in the broth. Bacterial growth was indicated by turbidity.

The checkerboard method: The checkerboard method was performed using 96-well microtitre plates as described previously (17; 20; 22), to obtain the FIC (Fractional inhibitory concentration) index. The microplate assay was arranged as follows: Essential oil A (EOA) was diluted two-fold along the x-axis, whilst EOB B was diluted two-fold along the y-axis. The final volume in each well was 100 µL comprising 50 µL of each EO dilution. Subsequently, 100 µL of media containing 2 × 10^6 CFU/mL of the indicator strain were added to all wells. The plates were then incubated at 30◦C or 37◦C for 18 h. The FIC indices were calculated as FICA+FICB, where FICA and FICB 18 are the minimum concentrations that inhibited the bacterial growth for essential oils A and B,

respectively. Thus, FICs were calculated as follows: FICA = (MICA combination/MICA alone) and FICB = (MICB combination/MICB alone). The results were interpreted as synergy (FIC < 0.5), addition (0.5 ≤ FIC ≤ 1), indifference (1 < FIC ≤ 4) or antagonism (FIC > 4). All experiments were done in triplicate.

F. Bioassays

Serial dilutions (V/V) of each essential oil were made in absolute alcohol. A series of five dilutions and an alcoholic control were selected for testing. Test concentrations varied from 40 to 90 ppm for essential oil.

Larvicidal effect of plant essential oils were tested according to the standard WHO protocol (23; 24), with slight modifications. 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 *Anopheles gambiae* batched for each dose tested and one control which consisted of 1 mL of absolute ethanol in 249 mL of distilled water. After a period of 24 h, 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, incoordination 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. Replicates with ≥15% mortality in the control were discarded from the analysis.

Ovicidal effect bioassays: 1 mL of test solution was mixed with 29 mL of distilled water in six plastic cups (of 115 mm diameter and 80 mm depth). Then, 20 recently-laid eggs were held in the insectary and egg mortality was scored 24 h post-treatment. Eggs that did not hatch after this period were considered as dead. A total of three replicate were carried out.

We considered criteria of Komalamisra et *al*. (25) and Bucker et *al*. (26) 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.

G. Statistical analysis

For comparison of MIC and FIC values, tests were made in triplicate. Analysis of variance was performed. The data were analyzed with Student's t-test or one-way ANOVA followed by Bonferroni test (GraphPad Prism 5.01; GraphPad Software Inc., San Diego, USA). Significant differences between means were determined by Fisher's test at the threshold of ($p < 0.05$).

III. RESULTS AND DISCUSSION

The essential oils air-dried leaves of *Eucalyptus citriodora, Cymbopogon giganteus* and *Cymbopogon nardus* medicinal plants were subjected to hydrodistillation using a Clevenger-type apparatus and the specific coloured oils were obtained in the yield of 6.86% (w/w), 10.00% (w/w) and 0.58% (w/w) for *E. citriodora, C. nardus* and *C. giganteus*, respectively.

A. Antioxidant activity

Table 1 show the DPPH radical inhibition effect of essential oils alone and in combination.

Table 1. Comparison of relative antioxidant activity and antiradical action.

EC = Eucalyptus citriodora, CG = Cymbopogon giganteus, CN = Cymbopogon nardus, AAI = Antioxidant Activity

Essential oils	Equations	R^2	DPPH : IC_{50} (µg/mL)	IAA	Activity
E citriodora	Y=1.2872X+1.9983	0.9378	34.2±2.3	1.16	Strong
C. nardus	Y=0.582X+0,3833	0.9979	85.25±0.5	0.46	Poor
C. giganteus	Y=0.7103X+3.1167	0.9579	66±0.1	0.6	Moderate
In combinaison					
EC-CN	Y=1.127X+0.5000	0.9992	4.32±0.6	11.57	Very strong
EC-CG	Y=1.855X+0.7887	0.9542	2.79±0.1	14.17	Very strong
CG-CN	Y=9.737X+1.659	0.9066	1.97±0.2	20. 46	Very strong
Vitamin C	Y=14.559X-0.613	0.9996	4.68	11.32	Very strong
BHT	Y=5.659X+11.513	0.9966	6.30	7.85	Very strong

Index, BHT= butylated hydroxytoluene: positive control.

The essential oils exhibited a DPPH free radical scavenging action. The IC_{50} values were 34.20; 66.00 and 85.25 µg/mL for essential oils of *E. citriodora, C. nardus* and *C. giganteus,* respectively. It showed a weak scavenging activity in comparison to the activities of vitamin C and BHT (IC_{50} values of 3.48 µg/mL and 6.30 µg/mL). The AAI of essential oils ranged from 0.46 to 1.06. The activities are poor, moderate and strong for *C. nardus, C. giganteus* and *E. citriodora,* respectively. The AAI of essential oils alone can be compared to AAI of vitamin C and BHT (AAI values of 11.32 and 7.85 respectively) while those of combinations ranged from 0.46 to 1.16. The paired combinations of *E. citriodora, C. giganteus* and *C. nardus* had very strong effects (AAI values ranged from 11.57 and 20.46). It showed a very strong scavenging activity in comparison to the activity of vitamin C and BHT. The strong DPPH radical scavenging action of essential oils alone and in combination, can be attributed to the presence of some components the antioxidant activity citronellal; citronellol; linalool, isopulegol, trans-paramentha- 1(7), 8-dien-2-ol, trans-para-mentha-2, 8-dien-1-ol, cis-paramentha- 2,8-dien-1-ol, a trans pipéritol, neral, myrcene and geraniol (11; 13; 15; 17). Essential oils have beneficial biological effects to scavenge free radicals. Free radical scavenging of essential oils is an important property underlying their various biological and pharmacological activities (18; 19; 27; 28; 29; 30).

B. Antimicrobial activity

The antimicrobial proprieties of *Eucalyptus citriodora, Cymbopogon giganteus* and *Cymbopogon nardus* essential oils alone and in combination were evaluated against a set of 11 microorganisms (Table 2).

Table 2. Antimicrobial activities of essential oils: inhibition zone diameters (mm).

Bacterial strains	Essential oils			In combinaison			Antibiotics		
	E. citriodora	*C. nardus*	*C. giganteus*	EC-CN	EC-CG	CG-CN	Te	Am	Gen
Bacillus cereus LMG 13569 BHI	16±0.5	14±2.0	16±0.3	21±1.5	18±2.0	33±1.5	18	ND	13
Echerichia coli 105182 CIP	11±0.7	17±0.7	24±0.5	28±2.4	28±2.1	24±1.1	7	ND	10
Enterobacter aeogenes CIP 104725	11±0.3	12±0.5	13±0.7	22±3.1	15±1.1	21±0.9	10	7	16
Enterococcus faecalis 103907CIP	15±0.0	23±3.0	24±2.0	28±1.2	26±1.0	36±1.2	19	7	30
Listeria monocytogenes CRBIP 13.134	12±0.2	18±2.0	25±2.0	34±2.0	14±0.5	30±1.2	14	7	13
Pseudomonas aeruginosa CRBIP19.249	15±0.4	0±0.0	21±0.4	0±0.0	0±0.0	0±0.0	20	7	21
Salmonella enterica CIP 105150	11±0.7	25±0.7	21±0.7	34±2.0	20±1.2	28±1.3	16	7	28
Salmonella typhimurium ATCC 13311	15±0.2	28±2.0	16±0.5	32±1.0	20±1.0	16±2.0	15	7	20
Shigella dysenteria 5451 CIP	10±0.2	27±2.0	11±0.5	36±2.0	20±1.0	11±0.5	16	ND	24
	30±2.8	25±1.7	31±0.5	24±1.4	17±2.3	14±1.3	17	ND	13
	Staphylococcus aureus ATCC 9144								
Streptococcus pyogenes	15±0.7	14±0.7	11±0.2	20±0.2	18±2.0	33±1.5	21	22	10

Am = Ampicillin (30 µg), Gen = Gentamicin (10 µg), Te = Tetracycline (30 µg), *EC = Eucalyptus citriodora, CG = Cymbopogon giganteus, CN = Cymbopogon nardus,* ND = Non determined.

Their potency was assessed qualitatively and quantitatively by the presence or absence of inhibition zones diameters (ZDs). The inhibition zone of essential oils varies from 10 to 30 mm for the bacteria. *Cymbopogon giganteus* essential oil showed antimicrobial effects against all microorganisms tested. It exhibited more activity on *Staphylococcus aureus* (31 mm), *Listeria monocytogenes* (25 mm), *Enterococcus faecalis* and *E. coli* (24 mm) than *Cymbopogon nardus* and *Eucalyptus citriodora*. The essential oil of *Eucalyptus citriodora* exhibited more activity on *Staphylococcus aureus* (17 mm) and *Bacillus cereus* (16 mm) than *Cymbopogon nardus*. *C. nardus* essential oil exhibited more activity on *Salmonella typhimurium* (28 mm), *Shigella dysenteria* (25 mm) and *Salmonella enterica* (23 mm) than *C. giganteus* and *E. citriodora* essential oils.

The paired combinations showed antibacterial activity against bacterial strains and presented an antimicrobial property stronger than essential oils alone (ZDs varies from 11 to 36 mm). Combinations of *E. citriodora* with *C. nardus* exhibited more activity on *Shigella dysenteria* (36 mm), *Listeria monocytogenes* (34 mm), *Salmonella enterica* (28 mm) and *Salmonella typhimurium* (32 mm) than combinations of *C. giganteus* with *C. nardus* or *E. citriodora*. Combination of *C. giganteus* with *C. nardus* was obtained best inhibition zone diameter for *Enterococcus faecalis* (36 mm), *Bacillus cereus* (33 mm) and *Streptococcus pyogenes* (33 mm). It is interesting to note that all the paired combinations present an antibacterial activity stronger than Ampicillin, Gentamicin and Tetracycline against microorganisms tested. The correlation between two essential oils different examined was generally larger zone diameter inhibition correlated.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The MICs and MBCs values summarized in table 3 confirmed the antibacterial activity of the plants. In all cases the MIC was equivalent to the MBC, indicating a bactericidal action of the essential oil. *Cymbopogon nardus* essential oil had bactericidal activity for all bacteria tested except *Pseudomonas aeruginosa* CRBIP 19249. *Cymbopogon giganteus* essential oil exhibited the best antibacterial property for *Echerichia coli* 105182 CIP (1 mg/mL), *Staphylococcus aureus* ATCC 9144 (2.5 mg/mL), *Bacillus cereus* LMG 13569 BHI (2 mg/mL), *Enterobacter aeogenes* CIP 104725 (4 mg/mL), *Salmonella enterica* CIP 105150 and *Salmonella typhimurium* ATCC 13311 (5 mg/mL). However, in the most cases the MIC was equivalent to the MBC and indicated a bactericidal action of *C. giganteus* essential oil. It was bacteriostatic effect for *Bacillus cereus* LMG 13569 BHI and *Streptococcus pyogenes.* Essential oil of *Eucalyptus citriodora,* shows an antibacterial action with MIC and MBC equal to 2.5 and 20 mg/mL on *Streptococcus pyogenes* (2.5 mg/mL), *Enterobacter aeogenes* CIP 104725 (4 mg/mL), *Enterococcus faecalis* 103907 CIP and *Staphylococcus aureus* ATCC 9144 (8 mg/mL). It showed a weak antimicrobial activity with MIC and MBC equal to 20 mg/mL of *Listeria monocytogenes* CRBIP 13134, *Salmonella typhimurium* ATCC 13311 and *Shigella dysenteria* 5451 CIP. *Eucalyptus citriodora* essential oil is bacteriostatic against *Bacillus cereus.* The results support previous data by Obame et *al*. (18; 19; 29; 30), who reported that essential oils exhibited both antibacterial activity against bacteria Gram negative and positive.

Interaction studies

The fractional inhibitory concentration (FIC) indices ranged from 0.03 to 1.94 for paired combinations of *Eucalyptus citriodora, Cymbopogon giganteus* and *Cymbopogon nardus* essential oils (Table 3). The interaction antagonism or addition, indifferent and synergy, between two oils, depend on the concentrations of the single component and the overall susceptibility of the target microorganism. All the paired combinations showed synergistic effects against *Echerichia coli* 105182 CIP, *Enterobacter aeogenes* CIP 104725, *Enterococcus faecalis* 103907 CIP and *Listeria monocytogenes* CRBIP 13134. An additive activity was observed for *Streptococcus pyogenes*. Combinations of *C. nardus* with *E. citriodora* or *C. giganteus* had synergistic effects on the inhibition of *Salmonella typhimurium* ATCC 13311 and *Staphylococcus aureus* ATCC 9144. Combinations of *C. giganteus* with *E. citriodora* or *C. nardus* had synergistic effects on *Salmonella enterica* CIP 105150 and additive action on *Shigella dysenteria* 5451 CIP. Combinations of *C. nardus* with *E. citriodora* had indifferent effects on *Bacillus cereus* LMG 13569 BHI and *Shigella dysenteria* 5451 CIP, and an additive effect was observed for *Salmonella enterica* CIP 105150. Combinations of *C. giganteus* with *C. nardus* showed synergistic effects against

Bacillus cereus LMG 13569 BHI. Combinations of *C. giganteus* with *E. citriodora* showed indifferent effects against *Bacillus cereus* LMG 13569 BHI and *Staphylococcus aureus* ATCC 9144. An additive action was observed for *Salmonella typhimurium* ATCC 13311. The efficacy of combinations appears to be related to chemical composition of combined essential oils and to possible interactions between their major components (11; 13; 15; 17; 18; 19; 31; 32).

Table 3. Antibacterial (MIC, MBC), Fractional Inhibitory Concentration (FIC) and interaction between essential oils.

Bacteria	Essential oils						In combinaison					
	E. citriodora		*C. nardus*		*C. giganteus*		EC-CN		CG-CN		CG-EC	
	MIC	MBC	MIC	MBC	MIC	MBC	FIC	Inter-action	FIC	Inter-action	FIC	Inter-action
Bacillus cereus LMG 13569 BHI	12	24	3	3	2	4	1.89	**I**	0.4	**S**	1.94	**I**
Echerichia coli 105182 CIP	16	16	0.5	0.5	1	1	0.1	**S**	0.4	**S**	0.4	**S**
Enterobacter aeogenes CIP 104725	8	8	2	2	4	4	0.25	**S**	0.06	**S**	0.03	**S**
Enterococcus faecalis 103907CIP	8	8	5	5	10	10	0.18	**S**	0.03	**S**	0.14	**S**
Listeria monocytogenes CRBIP 13134	20	20	4	4	8	8	0.06	**S**	0.45	**S**	0.25	**S**
Pseudomonas aeruginosa CRBIP 19249	>8	>16	8	>16	8	>8	ND	**ND**	ND	**ND**	ND	**ND**
Salmonella enterica CIP 105150	1	1	2.5	2.5	5	5	1	**Ad**	0.45	**S**	0.25	**S**
Salmonella typhimurium ATCC 13311	20	20	2.5	2.5	5	5	0.06	**S**	0.45	**S**	0.5	**Ad**
Shigella dysenteria 5451 CIP	20	20	8	8	16	16	2	**I**	0.75	**Ad**	0.52	**Ad**
Staphylococcus aureus ATCC 9144	8	8	2.5	2.5	2.5	2.5	0.17	**S**	0.4	**S**	2	**I**
Streptococcus pyogenes	2.5	2.5	2	5	10	25	1	**Ad**	0.8	**Ad**	0.8	**Ad**

EC = Eucalyptus citriodora, CG = Cymbopogon giganteus, CN = Cymbopogon nardus, S= synergism, Ad= addition, I= indifference, An = antagonism, ND = Non determined.

This may explain variation of interaction observed between combinations and strains. The present study has demonstrated the potential of the combination of *Eucalyptus citriodora, Cymbopogon giganteus* and *Cymbopogon nardus* essential oils to increase antibacterial activity. The best synergistic effects among essential oils were obtained with combinations. Results also showed that one or more synergistic components can produce the desired antibacterial effect.

C. Ovicidal and larvicidal activities against Anopheles gambiae

Eucalyptus citriodora, Cymbopogon giganteus and *Cymbopogon nardus* essential oils exhibited larvicidal and ovicidal activities by WHO (1; 23), standard protocol against 3rd and 4th instar larvae of field-collected mosquitoes vectors of human disease members of the *Anopheles gambiae* (Table 4).

Table 4. Ovicidal and larvicidal activities against *Anopheles gambiae*.

Essential oils	Ovicidal activity			Larvicidal activity		
	CL_{50} (µg/mL)	Equations	Activity	CL_{50} (µg/mL)	Equat.0ions	Activity
E. citriodora	37.88	Y = 0.9345X + 14.602 R^2=0.9317	Very effective	7.33	Y = 6.8168X + 0.0036 R^2=1	Very effective
C. nardus	35.75	Y = 1.1883X + 5.3763 R^2=0.9886	Very effective	107.77	Y = 0.7491X – 30.7037 R^2= 0.9392	Moderate
C. giganteus	75.3	Y = 3.1386X + 5. 8173 R^2=0.9786	Moderate	64.4	Y = 7.1304X – 9.7370 R^2= 0.9329	Moderate
In combinaison						
EC-CN	22.08	Y = 1.9058X + 7.91 R^2=0.9529	Very effective	9.94	Y = 3.8801X + 11.429 R^2=0.9098	Very effective
EG-CN	20.37	Y = 5.9018X + 4.62 R^2=0.9239	Very effective	41.37	Y = 1.8380X + 14. 219 R^2=0.9807	Very effective
EC-CG	13. 64	Y = 1.9058X + 9.73 R^2=0.9605	Very effective	27.39	Y = 1.5470X + 10. 149 R^2=0.9980	Very effective

EC = Eucalyptus citriodora, CG = Cymbopogon giganteus, CN = Cymbopogon nardus.

Ovicidal activity eggs of *Anopheles gambiae,* was demonstrated, the median lethal concentration (LC_{50}) value of *Anopheles gambiae* ranged between 13.64-75.3 µg/mL. LC_{50} of *Anopheles gambiae* larvae ranged between 7.33-107.77 µg/mL. *Eucalyptus citriodora* showed the highest activity against the *Anopheles gambiae* eggs (LC_{50}=37.88 µg/mL) and larvae (LC_{50}=7.33 µg/mL). Larvae were more susceptible than eggs. *Cymbopogon nardus* showed very efficacy action against *Anopheles gambiae* eggs (LC_{50}=35.75 µg/mL) and moderate effect against *Anopheles gambiae* larvae (LC_{50}=75.3 µg/mL). Eggs were more susceptible than larvae. Of the three plants, essential oils from *Cymbopogon giganteus* had the lowest activity *Anopheles gambiae* eggs (LC_{50}=107.77 µg/mL) and larvae (LC_{50}=64.4 µg/mL).
All the paired combinations showed very efficacy action and lethal activity against the *Anopheles gambiae* eggs and larvae. The differences in the observed LC_{50} values are likely to indicate that *Anopheles gambiae* eggs and larvae experience different levels of susceptibility to plant essential oils. The paired combinations present larvicidal and ovicidal activities stronger than essential oils. Combinations of *E. citriodora* with *C. nardus* or *C. giganteus* exhibited the best larvicidal action (7.33 µg/mL) and ovicidal activity (13.64 µg/mL), respectively. Eggs were more susceptible than larvae with combinations of *C. giganteus* with *C. nardus* or *E. citriodora.* The results support previous data by Obame et *al*. (19), who reported that combinations of essential oils extracted from resin of *Aucoumea klaineana, Canarium schweinfurthii and Dacryodes edulis* exhibited both ovicidal and larvicidal activities against *Anopheles gambiae*. Ahadji-Dabla et *al*. (32), showed that natural botanical biostop moustiquos and physiological changes had a high larvicidal activity against both strains of *Anopheles gambiae* and elicited a wide range of physiological changes. The larvicidal activity of selected plant essential oil against important vector mosquitoes: Dengue Vector, *Aedes aegypti* (L.), malarial vector, *Anopheles stephensi* (Liston) and filarial vector, *Culex quinquefasciatus* (Say) (Diptera*:* Culicidae) were tested against mature-stage larvae of *Anopheles funestus* (11; 33, 34, 35).

IV. CONCLUSION

The use of natural products of plant origin constitutes an alternative approach for malaria control and better efficacy for combating various infections and dry resistance. The result may be useful for the combinaisons of *Eucalyptus citriodora, Cymbopogon*

giganteus and *Cymbopogon nardus* essential oils against pathogen bacteria and *Anopheles gambiae* eggs or larvae. The present study showed that the essential oils from the leaves of *E. citriodora, C. giganteus* and *C. nardus* alone and in combinations have interesting antioxidant, antimicrobial and insecticidal properties. Essential oils are also known to have ovicidal and larvicidal properties against various insect species. They enhance their toxic potential when associated. These oils lead to an inhibition of growth regulators in insects. Their effectiveness in inhibiting the growth of bacteria and killing *Anopheles gambiae* larvae and eggs *in vitro* was demonstrated. The accessibility of these materials, as well as the absence of reported ecotoxicity, makes them promising models for the elaboration of new antioxidant, antimicrobial drugs and biological insecticides. It confirms the multiple uses of these plants for the treatment of many infectious diseases in Gabon.

Conflict of interest statement

We declare that we have no conflict of interest.

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