American Journal of Essential Oils and Natural Products

Available online at www.essencejournal.com

AJEONP American Journal of Essential Oils and Natural Products

AkiNik

ISSN: 2321-9114
AJEONP 2019; 7(1): 28-35

Received: 11-11-2018
Accepted: 15-12-2018

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Antibacterial activity of selected plant essential oils on airborne bacteria and mode of action on membrane integrity

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Abstract
The aim of this study was to investigate the antibacterial effect of six essential oils against airborne bacteria. Essential oils were obtained by hydrodistillation and analyzed by gas chromatography and gas chromatography-mass spectrometry. Antibacterial susceptibility was evaluated by agar disk diffusion and micro atmosphere methods. The minimum inhibitory concentration and the minimum bactericidal concentration were determined by the microdilution method in liquid medium. Measurement of the release of cellular material that absorbs at 260 nm and quantification of intracellular biomolecules in extracellular medium were carried out to investigate the antimicrobial mode of action of EOs. Overall, EOs in vapor-phase showed a better activity compared to the liquid phase. The MICs of EOs ranged from 0.04 to 3.12 mg/mL. The EOs of *Eugenia caryophylla*, *Citrus sinensis*, and *Cymbopogon citratus* presented a bactericidal effect on all the strains tested. Concentration of intracellular material released in extracellular medium ranged from 185.7 ± 2.75 ng/µL to 764.1 ± 3.46 ng/µL for DNA, 126.5 ± 2.33 ng/µL to 626.6 ± 17.25 ng/µL for RNA and 692.2 ± 6.22 ng/µL to 1231 ± 2.52 ng/µL for proteins. *Citrus sinensis* and *Eugenia caryophylla* EOs induced greater damage to the different bacteria produced by a greater release of biomolecules. This study demonstrates that the selected EOs have significant antimicrobial activity in both the liquid and vapor phase against the bacteria tested, acting on the cell surface and causing the disruption of the bacterial membrane. These EOs are interesting alternatives to conventional antimicrobials for the control of indoor microbial contaminants.

Keywords: essential oils, airborne bacteria, antibacterial activity, mode of action, cell membrane

1. Introduction
Problems of indoor air quality are recognized as important risk factors for human health in both developed and less developed countries. Indoor air is also important because populations spend a substantial fraction of time within buildings. In residences, day-care centers, retirement homes and other special environments, indoor air pollution affects population groups that are particularly vulnerable due to their health status or age. Microbial pollution involves hundreds of bacteria and fungi species that grow indoors when sufficient moisture is available. Exposure to microbial contaminants is clinically associated with respiratory symptoms, allergies, asthma and immunological reactions [1]. Given the severity of the precarious situation, a microbiological purification is imperative with the aim of preserving public health and that of buildings. Antimicrobial agents described as disinfectants are often alternatively used as sterilizing agents and hygienic products. They are most at times strong and toxic biocidal chemical products or antimicrobials that are applied on contaminated surfaces [2]. Disinfection using aerosols has long been used for it has the dual advantage of sterilizing both air and surfaces. However, it could be more dangerous than being effective depending on the chemicals used which are sometimes harmful to humans [3]. However, there is increasing consumer concern regarding the safety of the synthetic chemicals used to disinfect and their side effects. Much attention is being given throughout the world to minimize the use of synthetic antibacterial agents in the disinfection of a space. Therefore, there has been a great interest in identifying natural and safe antibacterial compounds from various natural sources.
Substitution of the usual products for essential oils (EO) will be an interesting approach by virtue of the fact that some studies have proven that EOs exhibit good antibacterial, antifungal, insecticidal, antioxidant and anti-inflammatory properties [4-7].

In 1963, research carried out by Pr Griffon's revealed that essential oils in atmospheric diffusion had the power to destroy in half an hour all the molds and staphylococci, and to divide by 50 the number of microbial colonies [3]. On a physical level, essential oils stimulate and modify our physiological states by acting directly on the central nervous system as well as a well-targeted olfactory atmosphere can bring a lot of benefits and have a lot of positive repercussions. Essential oils help to maintain a healthy atmosphere, fight against diseases and microbes, and maintain good concentration [3].

The choice of essential oils in this scientific approach is justified not only by their low toxicity but also by their antimicrobial, deodorizing and relaxing virtues. Indeed, many of these natural products have an adequate chemical composition and have the advantage of being significantly less toxic than other synthetic disinfectants for a comparable result [3].

Therefore, in this study, we evaluated the antibacterial potential of six essential oils against three dominant bacteria species, isolated in the central library of the University of Yaoundé I, as well as its mode of antibacterial action.

2. Materials and Methods

2.1 Bacterial strains used

Bacillus subtilis, Staphylococcus aureus and *Pseudomonas aeruginosa* used in this study were obtained inside the Central Library of the University of Yaoundé I. The isolates were identified on the basis of Gram's staining, motility, cultural characterization and biochemical screening-routine methods [8, 9].

2.2 Plant material

For essential oil extraction, six aromatic plants including *Citrus sinensis* (L.) Osbeck (Rutaceae), *Cymbopogon citratus* DC Stapf (Poaceae), *Eugenia caryophylla* (Spreng.) Bullock & S.G. Harrison (Myrtaceae), *Mentha* sp. cf. *piperita* L. (Lamiaceae), *Cananga odorata* Hook & Thomson (Annonaceae) and *Eucalyptus globulus* Labill (Myrtaceae) were harvested or purchased and identified at the National Herbarium of Cameroon. Essential oil extractions were carried out by hydrodistillation using a Clevenger apparatus [10].

2.3. Chemical analysis of essential oils

2.3.1 Essential oils analysis

Essential oils were analyzed by Gas Chromatography (GC) and Gas Chromatography coupled with Mass Spectrometry (GC-MS) as described by Kenmegne *et al.* [11].

2.3.2 Gas chromatography (GC)

Analysis was performed on a Varian gas chromatograph, model CP-3380, with flame ionization detector containing two silica capillary columns: HP5 J&W Agilent (5%-Phenyl-methylpolysiloxane) capillary column (30 m × 0.25 mm i.d. × 0.25 µm film) and Supelcowax 10 (polyethylene glycol) fused capillary column (30 m × 0.25 mm i.d. × 0.25 µm film); N_2 was the carrier gas at 0.8 mL/min; injection type 0.1 µL of pure sample, split ratio 1:100; injector temperature 220 °C, detector temperature 250 °C; temperature program 50-200 °C at 5 °C/min, then kept at 200 °C for 10 min. The linear retention indices of the components were determined relative to the retention times of a series of *n*-alkanes. The entire set up was coordinated by Chromeleon (version 7.4) software system that ensured its functioning and follow-up of the chromatographic analysis.

2.3.3 Gas chromatography-mass spectrometry (GC/MS). GC-MS

Analyses were performed using a Hewlett Packard 5890 II gas chromatograph, interfaced with a quadrupole detector (Model 5972) and equipped with a HP-5 MS capillary column (30 m × 0.25 mm, film thickness 0.25µm). Helium was the carrier gas, at a flow rate of 0.6 mL/min. Injector and MS transfer line temperatures were 220 °C and 250 °C, respectively. The oven program temperature was the same as that used in the GC-FID analyses. Diluted samples (10:100 in CH_2Cl_2, v/v) of 1 µL were injected manually and in a split mode (1:100). The MS was operated in the EI mode at 70 eV, in the m/z range 35–300; electron multiplier 1460 eV; scan rate, 2.96 scan/s.

2.3.4 Qualitative analysis

The identification of the constituents was assigned on the basis of a comparison of their relative retention indices, calculated with reference to a series of *n*-alkanes (C_9–C_{22}), and their mass spectra with those of the standards (for main components). Those found in the literature and supplemented by the NBS75K database and Wiley 7th NIST 2014 EPA/NIH Mass Spectral Library Upgrade (provided by Hewlett Packard with the GC/MS control and data processing software) [12, 13].

2.4 Antibacterial activities

2.4.1 Agar disk diffusion test

A standard disc diffusion method by NCCLS was used with modifications [14]. In each experiment, microorganisms were cultured at 37 °C for 24 hours and prepared to turbidity equivalent to McFarland standard No. 0.5. Then 100 µL of the suspension was spread on the test plate (Muller Hinton Agar) after which sterile discs (6 mm diameter) were impregnated with 10 µL of the essential oils (50 mg/mL) and placed on the surface of the test plate. Gentamicin® (2mg/mL) was used as the antibacterial reference control. Plates were subsequently incubated at 37 °C for 24 hours and zones of inhibition expressed inmillimeter (mm) was measured using a slide caliper. All tests were performed in triplicate.

2.4.2 Micro atmosphere method

This method allows the effect of volatile fractions ofthe EOs to be studied. This method is based on the evaluation of the inhibitory activity of the volatile fraction of the substance to be tested on a given microorganism at a given incubation temperature. Bacterial inocula were prepared and inoculated as described above (agar disk diffusion test). 6 mm sterile filter paper disk impregnated with 10 µL of each EOs were carefully deposited at the center of the lids of the Petri dishes and turned upside down without the essential oil (EO) being in contact with the inoculated agar. The dishes were tightly covered and incubated [15].

2.4.3 Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the antibacterial agents were determined using the microdilution reference method recommended by the Clinical Laboratory Standards Institute (CLSI) with slight modification [16]. 100 µL of sterile nutrient broth was dispensed into the 96-well microtiter plates. A serial double dilution of geometric ratio of 2 of the EOs was realized into the broth in the wells over a concentration range of 12.5 mg/mL to 0.02 mg/mL and 500 to 7.8 µg/Ml for

Gentamicin®. Overnight cultures of each strain were prepared in nutrient broth and the final concentration of each well was adjusted spectrophotometrically to 1.5×10^6 CFU/mL.
Positive control (nutrient broth + inoculum) and negative control wells (EO + nutrient broth) were included in each test. All tests were done in triplicate and incubated at 37ºC for 24 hours. The Minimal Inhibitory Concentration (MIC) was defined as the lowest concentration of samples at which the microorganism did not demonstrate growth [16].
The MBC was assessed by subculture as follows: 50 µL of the content of wells (unrevealed) corresponding to concentrations ≥ MIC were transferred unto 150 µL of fresh nutrient broth. The plates were incubated at 37 ºC for 24 hours. 40 µL of alamarblue (0.5%) was used to reveal bacterial growth in each well. The MBC was considered as the lowest concentration of each antibacterial substance that did not allow any noticeable color change from blue to yellow [17].

2.5 Integrity of the cell membrane

2.5.1 Measurement of the release of intracellular material that absorbs at 260 nm after different times of exposure

The measurement of the release of the cellular material that absorbs at 260 nm from *B. subtilis S. aureus* and *P. aeruginosa* cells was carried out in aliquots of 2 mL of the bacterial inocula in sterile peptone water (0.1 g per 100 mL) to which EO was added at the MIC concentrations. The mixture was incubated at 37 °C. After 0, 30 and 60 min, the cells were centrifuged at 3500×*g*, and the absorbance of the obtained supernatant was measured with a spectrophotometer [18]**.** Control flasks containing bacterial supernatant without EO treatments were tested similarly. Results were expressed in terms of absorbance at 260 nm in each interval with respect to the ultimate time.

2.5.2 Quantification of release of DNA, RNA and proteins

Effect of EOs on membrane integrity of bacteria cells has been confirmed by measuring the quantities of biomolecules (DNA, RNA, Proteins) released into extracellular medium using Nano Drop 1000spectrophotometer [19]. After determination of inhibition parameters as described above, the quantification of intracellular content was achieved after the determination of the MIC. With effect, 1µL of the well contents corresponding to the MIC was pipetted and dispense onto the lower optical pedestal and the lever arm mas closed after which the appropriate constants of the sample to be measured was chosen, and the software automatically calculated the nucleic acid and protein concentrations [19].
Control flasks containing bacterial supernatant without Eos treatments were tested similarly. Results were expressed in terms of concentration (ng/µL) of intracellular material release in extracellular medium.

2.6 Statistical analysis

Data were combined and analyzed by analysis of variance (ANOVA). The ANOVA was performed with SPSS software (version 23.). The significant differences ($p<0.05$) were estimated by Tukey and values were expressed as mean ± SD.

3. Results and Discussion

3.1 Chemical composition of essentials oils

The results of the chromatographic analysis are summarized in the Tables 1. The analysis of *Cymbopogon citratus* showed that the major components are geranial (33.76 %), neral (23.82%), nerol (8.47 %), geraniol (7.25%). These results are similar to those generally found in the literature. The work of Nyegue *et al*, 2017 [6] on the essential oil of *C. citratus* showed the main constituents to be: geranial (49.2 %), neral (34.3 %) and myrcene (5.9 %). Ndoye [20] showed that the *C. citratus* phenotypes of Lomié and Longtsimbi were similar to those of Batourie in citral levels. This result shows us that the type of compound present in high quantity and its content varies slightly depending on the location of harvest of plant material.
The analysis of *Mentha* sp. cf. *piperita* revealed the major components as piperitone (50.63%), β-phellandrene (20.90%) and menthyl acetate (19.29%). Piperitone was also obtained by Nyegue *et al*, 2014 [21] as a major compound of fresh leaves of *Mentha* sp. cf. *piperita* from Cameroon**,** which shows that the species used in this study has a different chemotype from that of the commonly used species, rich in menthol [22, 23]. This variability of major compound could be due to the geographical origin of the plant [24].
The analysis of *Cananga odorata* showed that it is composed mainly of linalool (49.1%), germacrene D (12.35%), Benzyl benzoate (8.0%) and *p*-methylanisole (7.6%). The chemical profile of *Cananga odorata* EO was in accord with earlier reports of Giang and Son, 2016 [25].
For *Citrus sinensis* major component is limonene (88.2%) followed by *trans*-limonene oxide (1.8%), linalool (1.3 %), and myrcene (1.2 %). An investigation in China revealed that limonene was observed as the dominant constituent (77.5 %) in the peel oil of sweet orange, followed by myrcene (6.3 %), α-farnesene (3.6 %) and γ-terpinene (3.3 %) [26]. The main compoundsin *C. sinensis* from Uganda and Rwanda were limonene (87.9 and 92.5 %), myrcene (2.4 and 2.0 %), α-pinene (0.5 and 2.4 %) and linalool (1.2 and 0.9 %) [27]. Limonene (90.16 % and 77.34 %) was the main compound in fresh and dried pericarps of*C. sinensis* in China. Monoterpene hydrocarbons were the most abundant fraction in the oils of three Kenyan *C. sinensis* varieties, *i.e*., Salustiana (96.9 %), Valencia (94.5 %) and Washington navel (92.7%) oils. In each essential oil, limonene, α-pinene, sabinene and α-terpinene were the major compounds [28, 29].
The major components for *Eucalyptus globulus* are α-pinene (49.3%), eucalyptol or 1,8-cineole (23.0%) and α-terpineol (5.7%). α-pinenebeing the main compound obtained is the same as that reported by Nyegue *et al*. 2017 [20]. Although, studies from Taiwan, Uruguay, Algeria, Burundi, Congo, Australia and Tunisia reported 1, 8-cineole as the major component inthe leaf oils of *Eucalyptus* [30].

Table 1: Chemical profile of essentials oils.

Compounds	LRI	Percentage (%)					
		C. odorata	*M.* cf. sp. *piperita*	*E. caryophylla*	*C. sinensis*	*E. globulus*	*C. citratus*
α-Pinene	940	-	0.6	-	0.5	49.3	2.4
Camphene	943	-	-	-	-	2.6	0.3
Benzaldehyde	963	0.1	-	-	-	-	1.8
Sabinene	967	0.2	-	-	0.2	0.3	-
β-Pinene	985	-	0.7	-	-	0.3	1.0
Myrcene	995	0.1	1.4	-	1.2	-	6.9
α-Terpinene	1011	-	-	-	-	0.6	-

p-Methylanisole -	1029	7.6	-	-	-	-	-
Limonene	1036	-	-	-	88.2	-	-
β-Phellandrene	1038	-	21.0	-	-	-	-
Eucalyptol (= 1,8-cineole)	1041	-	-	-	-	23.0	-
(*Z*)-β-Ocimene	1042	0.1	-	0.2	-	-	-
(*E*)-β-Ocimene	1049	0.1	0.2	-	-	-	0.3
cis-Sabinene hydrate	1062	-	0.1	-	-	-	-
Terpinolene	1075	-	-	0.2	-	0.4	1.0
p-Cymenene	1090	-	-	-	-	0.6	-
trans-Sabinene hydrate	1094	-	-	-	-	-	1.3
Linalool	1103	49.1	-	-	1.3	3.0	1.5
β-Thujone	1115	-	-	-	0.3	-	-
exo-Fenchol	1119	-	-	-	-	2.2	-
α-Campholenal	1128	-	-	-	-	0.4	-
cis-Pinene hydrate	1139	-	0.3	-	-	-	-
Camphor	1144	-	-	-	-	-	0.8
Eucarvone	1145	-	-	-	-	2.1	-
Citronellal	1151	-	-	-	1.8	-	0.3
Pinocarvone	1160	-	-	-	-	0.4	-
Borneol	1164	-	-	-	-	0.4	1.2
p-Mentha-1,5-dien-8-ol	1168	-	0.4	-	-	-	-
Menthol	1170	-	0.3	-	-	-	-
Terpinen-4-ol	1183	0.3	-	-	-	4.2	2.3
α-Terpineol	1186	0.3	-	-	-	5.7	-
Methyl salicylate	1192	0.6	-	-	-	-	-
trans-Carveol	1220	-	-	-	0.7	-	-
Citronellol	1229	0.2	-	-	-	-	-
Nerol	1238	-	-	-	0.3	-	8.5
Neral	1262	-	-	0.4	0.6	-	24.0
Geraniol	1271	1.5	-	-	-	-	7.3
Piperitone	1276	-	50.7	-	-	-	-
Geranial	1279	-	-	-	-	-	33.8
(*E*)-Anethol	1283	0.2	-	-	-	-	-
trans-Sabinyl acetate	1289	-	-	0.5	-	-	-
Menthyl acetate	1293	-	19.3	-	-	-	-
trans-Pinocarveol acetate	1305	-	-	-	0.7	-	-
α-Terpinyl acetate	1344	-	0.1	-	-	-	-
Citronellyl acetate	1352	-	-	-	0.3	-	-
neoiso-Dihydrocarveol acetate	1355	-	0.1	-	-	-	-
Eugenol	1361	-	-	70.0	-	-	-
1-Undecanol	1372	-	0.3	-	-	-	-
α-Copaene	1371	-	-	-	-	0.3	-
Geranyl acetate	1378	4.1	-	-	-	-	1.3
β-Cubebene	1391	-	0.2	-	-	-	0.3
β-Caryophyllene	1419	-	-	-	-	0.3	0.2
β-Ylangene	1424	2.4	-	-	-	-	-
Cinnamyl acetate	1436	0.2	-	-	-	-	-
α-Humulene	1446	-	0.7	-	-	0.6	-
trans-Carvyl propanoate	1447	-	0.3	-	-	-	-
γ -Muurolene	1456	1.0	0.1	-	-	-	-
Germacrene- D	1484	12.4	1.0	-	-	-	0.5
γ-Amorphene	1486	-	0.2	-	-	-	-
α-Muurolene	1497	2.5	0.1	5.2	-	0.3	-
γ-Cadinene	1510	0.2	-	0.7	-	-	-
δ-Cadinene	1521	1.0	0.1	-	-	-	0.2
α-Cadinene	1536	-	-	-	-	0.3	-
Eugenol acetate	1537	-	-	16.2	-	-	-
Caryophylleneoxide	1587	0.5	0.1	-	-	-	-
2-Acetylnaphtalene	1597	-	-	0.6	-	-	-
10-*epi*-γ-Eudesmol	1619	-	0.1	-	-	2.0	-
epi-α-Cadinol	1640	1.0	-	-	-	-	-
α-Cadinol	1653	2.1	-	-	-	-	-
(*E*,*E*)-Farnesol	1706	2.6	-	-	-	-	-
Benzyl benzoate	1766	8.0	-	-	-	-	-
(*E*,*E*)-Farnesyl acetate	1812	0.7	-	-	-	-	-
Total %		99.1	98.1	94.0	96.1	99.3	97.3

Legend: N°: elution order given on apolar column (HP-5); LRI: Linear Retention Index on apolar (HP-5); %: relative percentage; (−): not found

3.2 Antimicrobial test

The susceptibility pattern of the tested microorganisms to the EOs are indicated below (Tables 2, 3). The presence of inhibition zones after incubation revealed the activity of the EOs on the bacteria tested. It was noticed that in micro-atmosphere, inhibition zones ranged from 0.0 ± 0 mm to 60.33 ± 4.04 mm, whereas by the aromatogram method it is ranged from 0 ± 0 to 22.33 ± 1.15 mm for the EOs and from 29.66 ± 0.57 mm to 35.33 ± 0.57 mm for the reference antibiotic.

In microatmosp here, the most active EO was *C. citratus* with inhibition diameters of 35.33 ± 3.05 mm; 45.66 ± 3.21 mm and 60.33 ± 4.04 mm for *B. subtilis*, *S. aureus*, and *P. aeruginosa* respectively. The least active EO was *C. odorata*

which did not exhibit any activity on all strains used. On the other hand, the aromatogram method revealed the most active EO to be that of *C. sinensis* with diameters of 9.33 ± 0.57 mm; 22.33 ± 1.15 mm and 19.66 ± 0.57 mm for *B. subtilis, S. aureus*, and *P. aeruginosa*, respectively, and the least active to be that of *E. globulus* with diameters from 0 ± 0 mm to 10.33 ± 0.57 mm.

Concerning inhibition parameters, the results are shown in Table 4 below, it appears from the Table 4 that Gentamicin® showed activity on all the strains used with MICs ranged from 0.01 mg/mL to 0.03 mg/mL. All the EOs tested exhibited an activity with MICs ranged from 0.04 to 3.12 mg/m L. Hence, the EO of *E. caryophylla, C. cinensis*, and *C. citratus* presented a bactericidal effect on all the strains used.

An important characteristic of essential oils and their components is their hydrophobicity, which enable them to partition the lipids of the bacterial cell membrane and mitochondria, disturbing the cell structures and making them more permeable [31]. Extensive leakage from bacterial cells or the exit of critical molecules and ions will lead to death. It has already been shown that the antimicrobial activity of volatile compounds results from the combined effect of direct vapor absorption on microorganisms and indirect effect through the medium that absorbed the vapor [32]. The antimicrobial activities, in general, have been mainly explained through terpenes with aromatic rings and phenolic hydroxyl groups able to form hydrogen bonds with active sites of the target enzymes, although other active terpenes, as well as alcohols, aldehydes and esters can contribute to the overall antimicrobial effect of essential oils [33].

By analogy with other studies, these oils, like other essential oils, arecomposed of molecules belonging to the groups of terpenes and oxygenated molecules (aldehydes, phenols, alcohols, ketones) [34, 35]. The latter might provide their antibacterial power [36, 37]. The antibacterial activity of the oils might also be correlated, on one hand, by the proportions and the chemical structure of the aforementioned components.

Table 2: Results of sensitivity tests by micro atmosphere: Inhibition zone diameter ± sd (mm)

Microbial isolates	*C. odorata*	*E. caryophylla*	*C. citratus*	*M.* sp. cf. *piperita*	*E. globulus*	*C. sinensis*
Bacillus subtilis	0 ± 0 ^{a}	16.66 ± 1.4^{b}	35.33± 3.05^{c}	0 ± 0^{c}	0 ± 0^{c}	17.33±1.15^{c}
Staphylococcus aureus	0 ± 0 ^{a}	20.33 ± 1.15^{b}	45.66 ± 3.21^{b}	22.66 ± 1.52^{a}	17.66 ± 1.15^{b}	33.66 ± 1.15^{a}
Pseudomonas aeruginosa	0 ± 0 ^{a}	33.66 ± 0.86^{a}	60.33 ± 4.04^{a}	13.66 ± 1.52^{b}	20.66 ± 0.57^{a}	21.66 ± 2.08^{b}

Legend: The values that had the same letters were statistically identical while those with different letters were statistically different with a significance level P <0.05

Table 3: Results of sensitivity tests by aromatogram: Inhibition zone diameter ± sd (mm)

Microbial isolates	Gentamicin®	*Cananga odorata*	*Eugenia caryophylla*	*Cymbopogon citratus*	*Mentha* sp.cf. *piperita*	*Eucalyptus globulus*	*Citrus sinensis*
Bacillus subtilis	33.33 ± 0.57^{b}	12.66 ± 1.15^{a}	10.33 ± 1 ^{c}	16.33± 1.52^{a}	12.66 ± 1.15^{a}	0 ± 0 ^{c}	9.33 ± 0.57^{c}
Staphylococcus aureus	35.33 ± 0.57^{a}	0 ± 0 ^{b}	17 ± 0^{a}	17 ± 2^{a}	15 ± 1^{a}	10.33± 0.57^{a}	22.33 ± 1.15^{a}
Pseudomonas aeruginosa	29.66 ± 0.57^{c}	11.33 ± 0.57^{a}	13.66 ± 1.15^{b}	9.66 ± 1.15 ^{b}	8.33 ± 0.57^{b}	8.66 ± 0.57 ^{b}	19.66 ± 0.57^{b}

Legend: The values that had the same letters were statistically identical while those with different letters were statistically different with a significance level P <0.05.

Table 4: Results of sensitivity tests of essential oils on certain microbial isolates

Microbial isolates	Inhibitonpara meters (mg/ml)	Gentamicin®	*Cananga odorata*	*Eugenia caryophylla*	*Cymbopogon citratus*	*Mentha* sp. cf. *piperita*	*Eucalyptus globulus*	*Citrus sinensis*
Bacillus subtilis	MIC	0.03	6.25	0.39	0.19	1.56	0.78	0.39
	MBC	0.03	ND	0.39	0.19	3.125	6.25	0.78
	MBC/MIC	1	ND	1	1	2	8	2
Staphylococcus aureus	MIC	0.01	0.78	0.09	0.04	1.56	0.39	0.04
	MBC	0.01	3.125	0.09	0.04	3.125	1.56	0.09
	MBC/MIC	1	4	1	1	2	4	2
Pseudomonas aeruginosa	MIC	0.03	6.25	0.39	0.39	6.25	0.78	0.39
	MBC	0.03	ND	0.39	0.78	ND	6.25	0.78
	MBC/MIC	1	ND	1	2	ND	8	2

Legend: MIC: minimal inhibitory concentration; MBC: minimal bactericidal concentration; ND= Not Determined

2.6 Integrity of the cell membrane

The leakage of cytoplasmic membrane was analyzed by determining the release of cellular materials including DNA and RNA which absorbs at 260 nm into the bacterial suspensions. After following the release of biomolecules after 0 min, 30 min and 60 min, it was noticed in Figure 1 that all EOs used in this study had an effect on the cell membrane after 30 min of exposure. Damage to the membrane by EOs was marked by an increase of absorbance at 260 nm compared to the control without EOs which implies the release of biomolecules (DNA, RNA) into the extracellular medium. Loss of significant 260-nm-absorbing materials with increase of time interval, suggesting that nucleic acids and some protein were lost through a damaged cytoplasmic membrane. Similar studies were carried out by Bajpai *et al* [38].

To confirm effect of EOs on membrane of tested bacteria, a quantification of released biomolecule was done. The results are shown in Table 5 below. It was noticed that the control made up of untreated bacteria with EOs showed no intratracellular content (DNA RNA and proteins) in extracellular medium. Concentration of intracellular material released in extracellular medium varying from 185.7 ± 2.75 ng/µL.to 764.1 ± 3.46 ng/µL for DNA, 126.5 ± 2.33 ng/µL to 626.6 ± 17.25 ng/µL for RNA and 692.2 ± 6.22 ng/µL to 1231 ± 2.52 ng/µL for proteins. On all the bacteria exposed to the different EOs, the EOs of *Citrus sinensis* and *Eugenia caryophylla* induced greater damage to the different bacteria. This is materialized by the induction of an output of a larger quantity of the intracellular content.

The effects of EOs may be due to their components rich in terpenoids as well as a synergy between the different

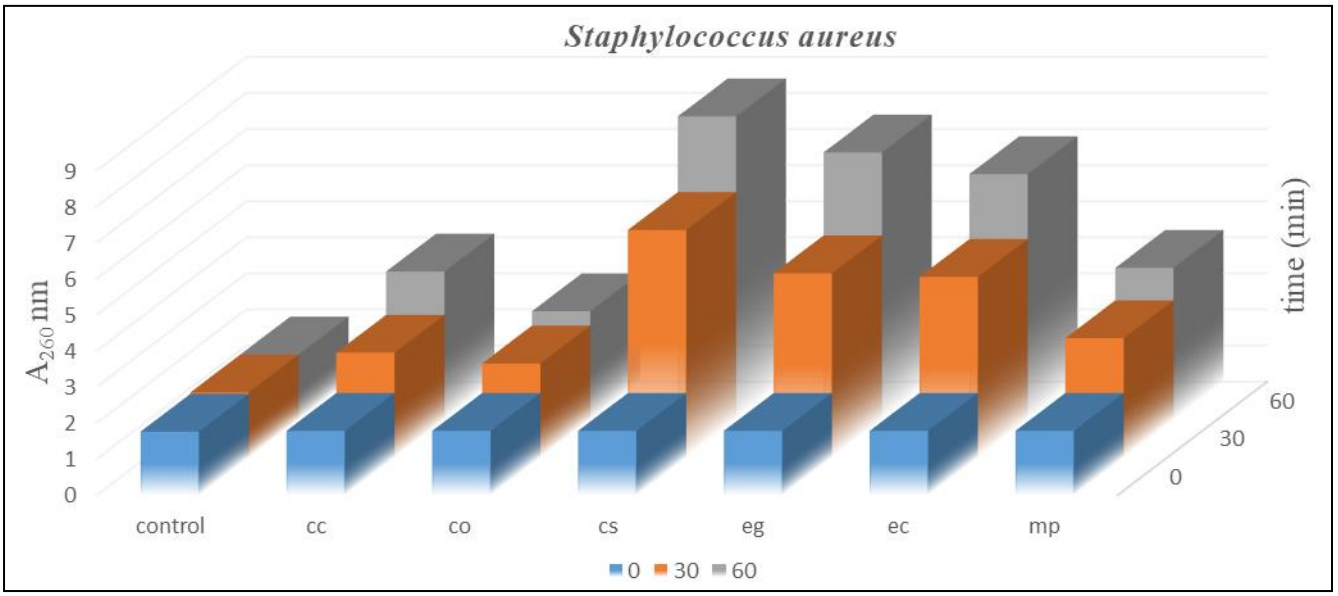
antimicrobial molecules contained in this EOs. The site of action of terpenoids is the cell membrane. Several monoterpenes were found to affect the structural and functional properties of the lipid fraction of the plasma membrane of bacteria and yeasts, causing intracellular materials to leak [39].

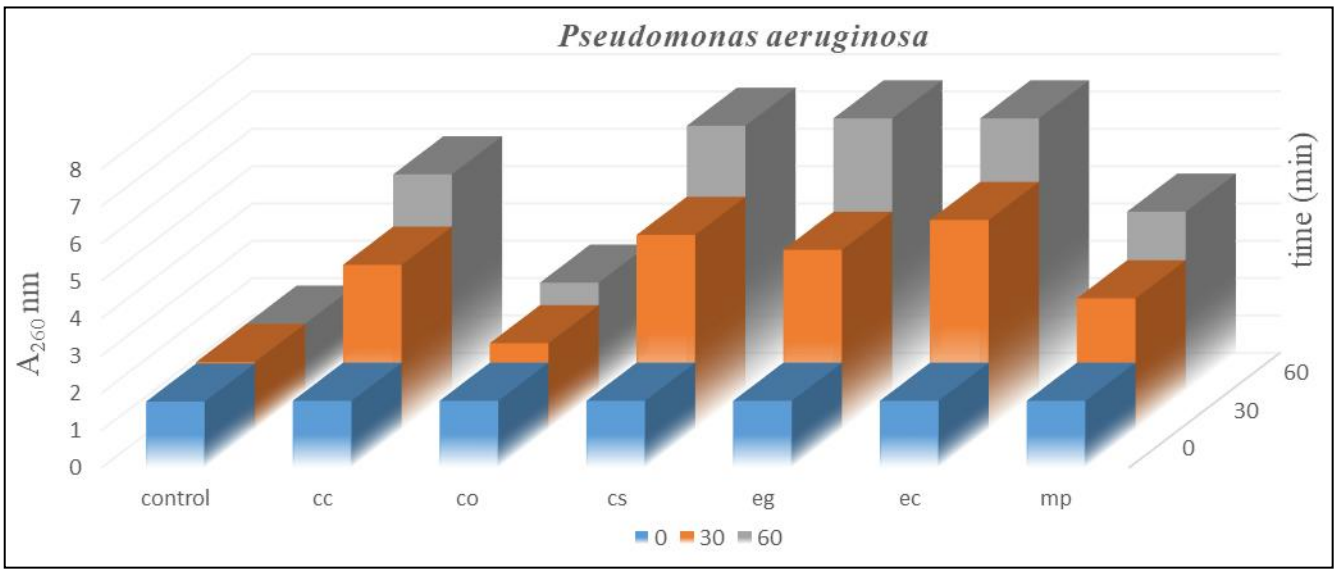
Release of intracellular components is a good indicator of membrane integrity. Small ions such as potassium and phosphate tend to leach out first, followed by large molecules such as DNA, RNA, and other materials, when treated with a suitable antimicrobial. Since these nucleotides have strong UV absorption at 260 nm, they are described as '260 nm absorbing materials' and this method is widely used in determining membrane integrity parameters [40, 41].

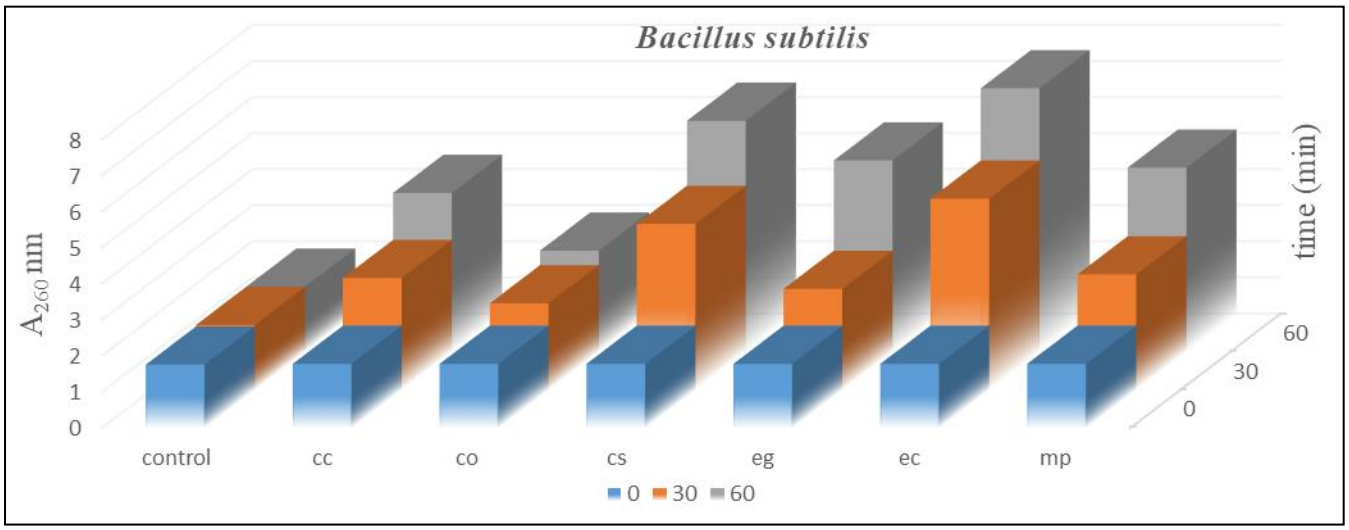
Table 5: Quantification of intracellular material in nanograms per microliter (ng/µL)

	Staphyloccocus aureus			*Pseudomonas aeruginosa*			*Bacillus subtilis*		
Conc (ng/µL)	**DNA**	**RNA**	**Protein**	**DNA**	**RNA**	**Protein**	**DNA**	**RNA**	**Protein**
Control	0 ± 0^{f}	0 ± 0^{d}	0 ± 0^{d}	0 ± 0^{f}	0 ± 0^{e}	0 ± 0^{e}	0 ± 0^{e}	0 ± 0^{g}	0 ± 0^{d}
CC	240.9 ± 16.75^{d}	186.9 ± 1.48^{c}	875.2 ± 10.23^{b}	336.3 ± 0.28^{d}	548.8 ± 17.60^{a}	851.3 ± 1.32^{c}	288.6 ± 1.83^{d}	219.2 ± 1.41^{d}	943.2 ± 2.32^{b}
CO	187.8 ± 2.12^{e}	155.1 ± 3.46^{c}	692.2 ± 6.22^{c}	185.7 ± 2.75^{e}	232 ± 7.83^{d}	622 ± 1.35^{d}	193.4 ± 0.28^{a}	126.5 ± 2.33^{f}	$886.9\pm 3.02^{b.c}$
CS	764.1 ± 3.46^{a}	626.6 ± 17.25^{a}	1231 ± 2.52^{a}	669.9 ± 5.79^{a}	$423.2\pm 2.12^{b.c}$	1125 ± 6.13^{a}	479.2 ± 10.81^{b}	384.2 ± 5.16^{b}	1062 ± 0.92^{a}
EG	385.8 ± 3.25^{b}	338.1 ± 13.22^{b}	$996.1\pm 7.23^{a.b}$	530.8 ± 6.01^{c}	$482.8\pm 2.19^{a.b}$	701.2 ± 5.32^{d}	368.6 ± 13.67^{c}	288.7 ± 0.77^{c}	823.6 ± 1.21^{c}
EC	189.6 ± 4.45^{e}	301 ± 1.55^{b}	$784\pm 1.94^{b.c}$	598.4 ± 3.67^{b}	$367.5\pm 5.95^{b.c}$	1054.6 ± 3.91^{b}	529.4 ± 8.55^{a}	429.1 ± 4.03^{a}	1092.1 ± 1.67^{a}
MP	302.1 ± 0.49^{c}	283.7 ± 4.08^{b}	704 ± 1.36^{c}	377.9 ± 39.66^{d}	$335.9\pm 1.34^{c.d}$	811.7 ± 2.41^{c}	353.9 ± 0.49^{c}	163.2 ± 6.36^{e}	934 ± 5.11^{b}

Legend: Conc: concentration ; CS: *Citrus sinensis*; CC: *Cymbopogon citratus*; EC: *Eugenia caryophylla*; MP: *Mentha* sp. cf. *piperita*; CO: *Cananga odorata*; EG: *Eucalyptus globulus.* The values that had the same letters were statistically identical while those with different letters were statistically different with a significance level $P < 0.05$.







Legend: cc: *Cymbopogon citratus*; co: *Cananga odorata*; cs: *Citrus sinensis*; eg: *Eucalyptus globulus*; ec: *Eugenia caryophylla*; mp: *Mentha* sp. cf. *piperita*

Fig 1: Effect of *C. citratus, C. odorata, C. sinensis, E. globulus, E. caryophylla* and *M.* sp.cf. *piperita* essential oils at MIC concentration on the release rate of material that absorbs at 260 nm from: *S. aureus, P. aeruginosa* and *B. subtilis*

4. Conclusions

Selected Eos was found to have a potential inhibitory effect on airborne bacteria. This effect of EO was associated with their ability to disrupt the bacterial membrane, causing loss of membrane integrity and leakage of 260-nm-absorbing material. These EOs might be considered as promising candidates for the development of natural antimicrobials for the control of indoor microbial contaminants.

5. Acknowledgments

Authors grateful the Cameroon National Herbarium and the Institute of Biomolecules Max Mousseron (France) for their technical support.

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