Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2022; 11(1): 23-31
Received: 06-11-2021
Accepted: 07-12-2021

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Effects of the chemical composition of essential oils from seven plants used in traditional medicine in Benin on the growth of eleven pathogenic bacteria in antimicrobial control

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DOI: https://doi.org/10.22271/phyto.2022.v11.i1a.14317

Abstract
The uncontrolled use of antimicrobials leads to an increase in the resistance of bacteria which becomes a public health problem.
To overcome this problem, our study aims to establish a link between chemical composition and antimicrobial activity and then evaluate cytotoxicity, of seven essential oils.
Antimicrobial activity of essential oils was assessed by macrodilution and solid-medium diffusion method on agar, then cytotoxicity test was evaluated *in vitro* by MTT method.
Results showed that essential oils of *Cymbopogon schoenantus*, *Cymbopogon giganteus*, *Cymbopogon citratus* and *Citrus aurantifolia* are the most bactericidal. Analysis of antimicrobial activity and chemical composition reveal that the essential oil of *Eucalyptus camaldulensis*, the least oxygenated (14.9%), is the least active. The other essential oils, which are more active, are all rich in oxygenated compound (28.4% to 87.0%). The cytotoxicity assessment shows that our essential oils are less cytotoxic than camptothecin.

Keywords: Antimicrobial activity, toxicity, chemical composition, medicinal plants, essential oils, Benin

Introduction
Antimicrobials are used to slow down growth or kill bacteria that cause infections and diseases. But resistance is the natural consequence of the use of antimicrobials which kill sensitive microorganisms and allow resistant strains to survive and multiply, posing a threat to global health [1]. It is responsible for an increase in morbidity, mortality and the length of hospitalization [2]. The World Health Organization (WHO) recently published a report on antimicrobial resistance [1]. Faced with this problem, several studies have been carried out to develop alternative efficient molecules against these infectious diseases. Medicinal plants are a source of bioactive molecules that could be exploited in the therapy of infectious diseases [3]. Essential oils have been schown to be effective in controlling the spread of certain bacterial agents [3, 4].
In Benin, more than 80% of the population uses plants for care needs [5]. *Psidium guajava*, *Eucalyptus camaldulensis*, *Citrus aurantifolia*, *Cymbopogon citratus*, *Cymbopogon giganteus*, *Cymbopogon nardus* and *Cymbopogon schenantus* are medicinal plants from the Beninese flora used by the population for antimicrobial control [5, 6]. In literature, several authors have indicated that these plants are used in antimicrobial control [6, 7, 8, 9, 10, 11, 12, 13].
This work aims to evaluate the antimicrobial activity of eleven pathogenic bacterial strains, along with cytotocycity of two cells strains of essential oils obtained from these plants.

Materials and Methods
Plant material
Cymbopogon citratus **(**DC) Stapf, *Cymbopogon giganteus* (Chiov**),** *Cymbopogon nardus* (L.) Rendle, *Cymbopogon schoenantus* (L.) Spreng, *Eucalyptus camaldulensis* Dehnh (Myrtaceaes), *Psidium guajava* Linn (Myrtaceaes) and *Citrus aurantifolia* (Christm.) Swingle (Lime) were collected, from the Botanical Garden of the Abomey-Calavi University and identify at the University of Abomey-Calavi Herbarium.

Microorganism's cultures
Eleven bacterial strains including five Gram positive bacteria (*Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* T22695, *streptococcus oralis*, *Micrococcus luteus* ATCC

10240 and *Enterococcus faecalis* ATCC 29212) and six Gram negative bacteria (*Escherichia coli* ATCC 25922, *Escherichia. coli* O157H7, *Pseudomonas aeruginosa* ATCC 27853, *Proteus mirabilis* A24974, *Proteus vulgaris* A25015 and *Salmonella typhi* R309514021) were used in this study.

Parasites, cell lines and media

The macrophage-like cell line, CHO Chinese Hamster Ovary cells (ATCC N° CCL-61, batch 4765275), were cultivated *in vitro* in Ham's F12 Nutrient Mixture 21765 medium (Gibco) containing 2 mM L-glutamine supplemented with 10% heat-inactivated foetal bovine serum (Gibco) and penicillin-streptomycin (100 UI/mL to 100 µg/mL). The human non cancer fibroblast cell line, WI38 (ATCC N° CCL - 75 from LGC Standards) was cultivated *in vitro* in DMEM medium (Gibco) containing 4 mM L-glutamine, 1 mM sodium pyruvate supplemented with 10% heat-inactivated foetal bovine serum (Gibco) and penicillin-streptomycin (100 UI/mL to 100 µg/mL).

Chemicals and drugs

The following chemicals used

Dulbecco's Modified Eagle Medium (DMEM) and Ham's F12 culture media were purchased from Life technologies corporation (Grand Island, NY 14072, USA); tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium-bromide) (MTT), (S)-(+)-camptothecin, dimethyl sulfoxide (DMSO) and *n*-alkanes "C7-C28" were obtained from Sigma-Aldrich (Steinhein, Germany), and Fluka Chemie (Buchs, Switzerland). All compounds were of analytical standard grade. Ter-Butyl methyl ether (TBME) was an analytical grade solvent purchased from Fluka Chemie, and anhydrous Na_2SO_4 was of analytical reagent grade from UCB (Brussels, Belgium).

Isolation of essential oils

About 500 g of each fresh plants material were extracted in steam distillated for 3 h [14] and the oils obtained were stored at 4 °C. The essential oil yields were calculated based on the fresh plant material [15].

Chemical analysis of essential oils

GC/MS analysis

GC/MS analysis was carried out using a TRACE GC 2000 series (Thermo-Quest, Rodano, Italy), equipped with an autosampler AS2000 Thermo-Quest. The GC system was interfaced to a Trace MS mass spectrometer (ThermoQuest) operating in the electronic impact mode at 70 eV. HP 5MS column (30 m × 0.25 mm, film thickness: 0.25 µm) was used; injection mode: splitless; injection volume: 1 µL (TBME solution); split flow: 10 mL/min; splitless time: 0.80 min; injector temperature: 260 °C; oven temperature was programmed as following: 50 to 250 °C at 6 °C/min and held at 250 °C for 5 min; the carrier gas was helium with a constant flow of 1.2 mL/min. The coupling temperature of the GC was 260 °C and the temperature of the source of the electrons was 260 °C. The data were recorded and analyzed with the Xcalibur 1.1 software (ThermoQuest) [15].

Identification of oils components

Components of volatile oils were identified by comparison with those of commercial EI-MS spectra library [16, 17], home-made mass spectra library made from pure substances and components of known oils. Mass spectrometry literature data were also used for the identification [18]. Quantification (expressed as percentages) was carried out by the normalization procedure using peak areas obtained by FID. Values are expressed as mean ± standard deviation (n = 3).

Antimicrobial activity

Essential oils emulsion

40 µL of essential oil and 20µL of Tween 80 are added to 2 mL of Mueller Hinton medium, all introduced into a hemolysis test tube and homogenized. The mixture thus obtained has a concentration of 20 mg/mL.

Sensitivity test

The agar perforation method inspired from those of Dah-Nouvlessounon *et al.* [19] was used to screen for antimicrobial activity. Fifty microliter of essential oils extract solution (20 mg/mL) was aseptically lodged in the hole. These dishes were incubated at 37 °C for 24 and 48 h. Each sample was used in triplicate for the determination of antibacterial activity.

Determination of Minimum Inhibitory Concentrations (MIC) and Bactericidal Concentration (MBC)

The Minimum Inhibitory Concentrations (MIC) of essential oils extract was performed by macrodilution method. Nine (9) dilutions of 1 mL of the oils emulsion were performed to obtain the concentrations of 10 mg/mL, 5 mg/mL, 2.5 mg/mL, 1.25 mg/mL, 0.625 mg/mL, 0.312 mg/mL, 0.156 mg/mL, 0.078 mg/mL and 0.039 mg/mL in screw caped. To 1 mL of the above concentrations was added 1 mL of the bacteria inoculum (10^6 UFC/mL) to obtain 2 mL as a final volume. Culture medium without samples and others without microorganisms were used in the tests as control. Tubes were incubated at 37 °C for 18-24 hours and growth was indicated by turbidity. The MIC is the lowest concentration of the compound at which the microorganism tested does not demonstrate visible growth (turbidity) [19].

The MBC was determined by solid medium culture of all of the tubes from the MIC to high concentrations. These dishes were incubated at 37 °C for 24 h. The highest dilution that yielded no bacterial growth on solid medium was taken as MBC [19].

Cytotoxicity assay

Cytotoxicity of the oils against CHO and WI38 cells was evaluated as described previously by Kpadonou *et al.* [20]. Cytotoxicity were made at least in duplicate. GraphPad Prism 4.0, was used for statistical analysis. Statistical significance was set at $P<0.05$.

Results and discussion

Chemical composition of the essential oils

Yields of seven essential oils varying between 0.65% to 1.88% then group together in Table 1. *C. schoenantus* (1.88%) has the highest yield followed by *E. camaldulensis* (1.38%), *C. aurantifolia* (1.28%), *C. nardus* (1.06%), *P. guajava* (0.78%), *C. citratus* (0.71%) and *C. giganteus* (0.65%) the lowest yield. *C. schoenantus* is 1.5 times richer in EO (essential oils) than *C. nardus* and 2.5 times richer than *C. citratus* and *C. giganteus*.

Yield of *E. camaldulensis* (1.38%) and *P. guajava* (0.78%), confirms the work of [20]. The leaves of *C. aurantifolia* gave an oil yield (1.28%) lower than that obtained in Brazil at Lavras (2.2%) by [12]. For the genus *Cymbopogon*, the yield obtained for *C. schoenantus* (1.88%) is much lower than those obtained for the same plant in Benin, in Djougou (3.49%; [6]), in Boukounbe (2.8%; [21]) for dried leaves. On the other hand,

the yield of *C. nardus* (1.06%) is lower than that obtained in Benin at Abomey-Calavi (6.88%; [22]). Similarly, the yield of *C. citratus* (0.71%) is much lower than those obtained for the same plant in Benin at Abomey-Calavi (4.31%; [22]), at Akogbato (1, 7%; [21]) for dried leaves. The yield of *C. giganteus* (0.65%) is lower than those obtained for the same plant in Benin at Houintopka (0.91%; [6]), at Koudo (1.4%; [21]) for dried leaves. The difference of yield could be explained by the influence of the place, the season, the time of harvest in the day or the vegetative stage of the plant [20, 23, 24].

In Table 1, the analysis of the chemical composition of essential oils is summarized. EO of genus *Cymbopogon* and *C. aurantifolia* are richer in oxygenates (61.1% to 87.0%) than in hydrocarbons, and the genus *Cymbopogon* comes first. However, *E. camaldulensis* and *P. guajava* contain more hydrocarbon compounds (54.7% to 78.1%). Sesquiterpenes constitute the major chemical group in *P. guajava* (Table 1).

C. giganteus was characterized by the *trans*-*p*-mentha-1 (7), 8-dien-2-ol (18.3 ± 0.17%) accompanied by *trans*-carveol (17.4 ± 0.16%), *trans*-*p*-mentha-2,8-dienol (15.5 ± 0.15%), *cis*-*p*-mentha-2,8-dienol (11.3 ± 0.03%), limonene (8.3 ± 0.08%), *cis*-carveol (7.3 ± 0.07%). In literature that the EOs of *C. giganteus* are characterized by the presence of molecules with a menthane skeleton, in particular *p*-menthadienols. This is observed in the EO of the plant in Benin [6], Burkina Faso [10], Togo [25].

The Citral (geranial (39.5 ± 0.00%) and neral (35.5 ± 0.15%)) is the major compound in the oil of *C. citratus* accompanied by β-Pinene (10.1 ± 0.04%). Previous work has shown that the EO of *C. citratus* acclimatized in Benin, consists mainly of citral (from 70 to 90%) which is the mixture of two geometric isomeric aldehydes: (31.2%) neral and (44, 5%) geranial [26], (33%) neral and (41.3%) geranial [21], (33.49%) neral and (36.64%) geranial [27]. In Congo: (33.33%) neral and (45.95%) geranial [28], in China: (32.58%) neral and (42.16%) geranial [29], in England: (38.349%) neral and (50.5%) geranial [30], in South Africa: (28.26%)) neral and (40.55%) geranial [31], in Brazil: (36.37%) neral and (53.2%) geranial and in Cuba: (35.21%) neral and (51.14%) geranial [32], in Burkina Faso: (34.6%) neral and (48.1%) geranial [10]. However, except the citral which is found mainly in several EO of *C. citratus*, another chemotype of this EO from Iceland, with as majority compounds citronellal (45.09%), citronellol (19.11%) and geraniol (13.57%) were reported in literature [33].

Table 1: Chemical composition, groups and yield of essential oils (mean ± sd, n = 3).

Compounds	KI	*C. citratus*	*C. giganteus*	*C. nardus*	*C. schoenantus*	*E. camaldulensis*	*P. guajava*	*C. aurantifolia*
β-pinene[c,h]	996	10.1±0.04	–	–	–	–	–	2.0 ± 0.03
(*p*)-2-Carene[c,h]	1005	–	–	–	13.0±0.20	–	–	–
p-cymene[c,h]	1023	–	–	–	–	18.2±0.02	–	–
Limonene[c,h]	1028	–	8.3 ± 0.08	2.2 ± 0.02	6.4 ± 0.10	1.8 ± 0.02	–	22.7 ± 0.01
1,8-cineole[c,o]	1033	–	–	–	–	7.5 ± 0.07	–	–
Ocimene[c,h]	1050	–	–	–	–	–	–	3.4 ± 0.03
γ-terpinene[c,h]	1062	–	–	–	–	57.1±0.04	–	–
Terpinolene[c,h]	1088	–	–	–	–	1.0 ± 0.01	–	–
β-linalol[c,o]	1101	1.0 ± 0.00	–	–	–	–	–	2.0 ± 0.04
trans-p-mentha-2,8-dienol[c,o]	1120	–	15.5±0.15	–	1.8 ± 0.03	–	–	–
cis-*p*-mentha-2,8-dienol[c,o]	1133	–	11.3±0.03	–	1.3 ± 0.02	–	–	–
Citronellal[c,o]	1153	–	–	–	–	–	–	3.6 ± 0.03
Verbenol[c,o]	1164	–	–	–	–	–	–	1.8 ± 0.03
trans-*p*-mentha-1(7),8-dien-2-ol[c,o]	1181	–	18.3±0.17	–	–	–	–	–
terpinene-4-ol[c,o]	1182	–	–	–	–	7.4 ± 0.07	–	–
β-citronellal[c,o]	1192	–	–	35.9±0.34	–	–	–	–
cis-verbenol[c,o]	1199	1.7 ± 0.01	–	–	–	–	–	2.2 ± 0.02
trans-4,5-epoxycarane[c,o]	1201	–	1.5 ± 0.01	–	–	–	–	–
cis-*p*-mentha-1(7),8-dien-2-ol[c,o]	1206	–	8.9 ± 0.08	–	–	–	–	–
cis-carveol[c,o]	1227	–	7.3 ± 0.07	–	–	–	–	–
β-citronellol[c,o]	1244	–	–	11.6±0.11	–	–	–	–
trans-carveol[c,o]	1246	–	17.4±0.16	–	–	–	–	–
trans-geraniol[c,o]	1249							1.5 ± 0.01
cis-carvone[c,o]	1267	–	3.4 ± 0.03	–	–	–	–	–
Neral[c,o]	1268	35.5±0.15	–	–	–	–	–	19.3 ± 0.00
cis-geraniol[c,o]	1291	4.3 ± 0.02	–	–	–	–	–	1.0 ± 0.02
Nerol[c,o]	1294	–	–	24.3±0.23	–	–	–	–
Piperitone[c,o]	1296	–	–	–	60.3±0.92	–	–	–
Geranial[c,o]	1328	39.5±0.00	–	–	–	–	–	20.5 ± 0.02
acetate de geranyle [c,o]	1340	1.0 ± 0.00	–	1.3 ± 0.01	–	–	–	6.9 ± 0.01
acetate de neryle [c,o]	1350	–	–	–	–	–	–	2.3 ± 0.03
β-elemene[c,h]	1353	–	–	1.9 ± 0.02	–	–	–	–
α-copaene[d,h]	1379	–	–	–	–	–	1.0 ±0.02	–
β-caryophyllene[d,h]	1394	–	–	–	–	–	8.1 ± 0.03	–
Helifolene[d,h]	1406	–	–	–	–	–	1.1 ± 0.02	–
Caryophyllene[d,h]	1415	–	–	–	–	–	–	2.4 ± 0.01
α-cedrene[d,h]	1418	–	–	–	–	–	1.0 ± 0.02	–
α-humulene[d,h]	1443	–	–	–	–	–	1.3 ± 0.02	–
(*E*)-β-farnesene[d,h]	1458	–	–	–	–	–	1.0 ± 0.01	–
β-santalene[d,h]	1460	–	–	–	–	–	1.1 ± 0.02	–
α-acoradiene[d,h]	1464	–	–	–	–	–	2.9 ± 0.05	–

α-neocallitropsene[d,h]	1475	–	–	–	–	–	1.7 ± 0.01	–
germacrene-D[d,h]	1477	–	–	1.5 ± 0.01	–	–	–	–
ar-curcumene[d,h]	1483	–	–	–	–	–	12.3±0.02	–
β-selinene[d,h]	1485	–	–	–	–	–	1.2 ± 0.00	–
(*Z*)-α-bisabolene[d,h]	1491	–	–	–	–	–	1.3 ± 0.02	–
α-selinene[d,h]	1492	–	–	–	–	–	1.2 ± 0.02	–
β-bisabolene[d,h]	1509	–	–	–	–	–	14.4±0.03	–
β-sesquiphellandrene[d,h]	1516	–	–	–	–	–	3.0 ± 0.05	–
(*E*)-γ-bisabolene[d,h]	1521	–	–	–	–	–	2.1 ± 0.03	–
δ-cadinene[d,h]	1523	–	–	1.1 ± 0.01	–	–	–	–
Elemol[d,o]	1556	–	–	9.0 ± 0.08	4.9 ± 0.08	–	–	–
(*E*)-nerolidol[d,o]	1564	–	–	–	–	–	2.4 ± 0.04	–
Cubenol[d,o]	1579	–	–	1.8 ± 0.02	–	–	–	–
oxyde de caryophyllene[d,o]	1581	–	–	–	–	–	2.2 ± 0.03	–
epi-cubenol[d,o]	1627	–	–	–	–	–	1.1±0.02	–
τ-eudesmol[d,o]	1630	–	–	–	1.1 ± 0.02	–	–	–
β-acorenol[d,o]	1634	–	–	–	–	–	2.2 ± 0.03	–
gossonorol[d,o]	1638	–	–	–	–	–	1.5 ± 0.02	–
τ-cadinol[d,o]	1639	–	–	1.1 ± 0.01	–	–	–	–
allo-aromadendrene epoxyde[d,o]	1640	–	–	–	–	–	–	–
β-eudesmol[d,o]	1648	–	–	–	3.1 ± 0.05	–	–	–
α-cadinol[d,o]	1654	–	–	2.0 ± 0.02	–	–	2.2±0.03	–
selin-11-en-4-α-ol[d,o]	1660	–	–	–	–	–	2.0±0.03	–
β-bisabolol[d,o]	1671	–	–	–	–	–	11.4±0.08	–
α-bisabolol[d,o]	1683	–	–	–	–	–	3.4 ± 0.06	–
14-hydroxy-α-humulene[d,o]	1714	–	–	–	–	–	–	–
4,4-dimethylandrost-5-en-3-one[e,o]	2184	–	1.7 ± 0.02	–	–	–	–	–
Total		93.1±0.22	93.6±0.80	93.7±0.88	91.9±1.42	93.0±0.23	83.1±0.71	91.6 ± 0.29
Yield (%)		0.71 ± 0.02	0.65 ± 0.02	1.06 ± 0.10	1.88 ± 0.12	1.38±0.02	0.78±0.02	1.28±0.03
Hydrocarbon compound		10.10±0.04	8.3 ± 0.08	6.7 ± 0.06	19.4±0.3	78.1±0.09	54.7±0.37	30.5± 0.08
Oxygenated compound: Oxygenated monoterpenes		83.0±0.18	85.3±0.72	73.1±0.69	63.4±0.97	14.9±0.14	–	58.7 ± 0.20
Oxygenated compound: Oxygenated sesquiterpenes		–	–	13.9±0.13	9.1±0.15	–	28.4±0.34	2.4 ± 0.01
Oxygenated compound: Total		83.0±0.18	85.3±0.72	87.0±0.82	72.5±1.12	14.9±0.14	28.4±0.34	61.1 ± 0.21
Monoterpenes		93.1±0.22	91.9±0.78	77.2±0.73	82.8±1.27	93.0±0.23	–	89.2 ± 0.28
Sesquiterpenes		–	–	16.5±0.15	9.1±0.15	–	83.1±0.71	2.4 ± 0.01
Others		–	1.7 ± 0.02	–	–	–	–	–

C. = Cymbopogon, E. camaldulensis = Eucalyptus camaldulensis, P. guajava = Psdium guajava, C. aurantifolia = Citrus aurantifolia, [c] = monoterpenes; [d] = sesquiterpenes; [e] = non terpenes; [h] = hydrocarbons; [o] = oxygenated; (-) = absence; (±) = standard deviation of three separate experiments.

More than six compounds show a percentage between (1 to 2%) in the oil of *C. nardus* with β-citronellal (35.9 ± 0.34%) accompanied by nerol (24.3 ± 0.23%), β-citronellol (11.6 ± 0.11%), elemol (9.0 ± 0.08%), as the majority compounds. This composition is close to that with the citronellal chemotype, geraniol and citronellol, other studies reported [28, 34, 35, 36].

In the EO of *C. schoenanthus* predominates piperitone (60.3 ± 0.92%) accompanied by (*p*) -2-carene (13.0 ± 0.20%), limonene (6.4 ± 0.10%), elemol (4.9 ± 0.08%). This composition more closely resembles that of the piperitone chemotype, obtained by other authors [11, 36].

No component of the EO *P. guajava* exceeds 15%. The major compounds were β-bisabolene (14.4 ± 0.03%), *ar*-curcumene (12.3 ± 0.02%), β- bisabolol (11.4 ± 0.08%) and β-caryophyllene (8.1 ± 0.03%).

These results this composition were different from that obtained for the same plant in Brazil [37] and in Costa Rica [38].

In *E. camaldulensis* predominates γ-terpinene (57.1 ± 0.04%) accompanied by *p*-cymene (18.2 ± 0.02%), 1,8-cineole (7.5 ± 0.07%), terpinen -4-ol (7.4 ± 0.07%). The composition of this oil is similar to that previously described in Calavi [20] but different from those studied in Egypt [39], Argentina [40], in Brazil [41], in Tanzania [42], which are richer in eucalyptol, *p*-cymene, spathulenol, cryptonne or in 1,8-cineole. This work confirms those of [20] who showed that the oil of the plant acclimatized in Benin is characterized by a low content of 1,8-cineole and by the absence of cryptone.

The *C. aurantifolia* oil contains a majority of limonene (22.7 ± 0.01%) accompanied by (*trans*) citral (20.5 ± 0.02%), (*cis*) citral (19.3 ± 0.00%), geranyl acetate (6.9 ± 0.01%). This composition is different from those obtained by [12, 43] who all reported limonene chemotype oils with higher rate than that obtained for EO.

In the literature, the variation in chemical composition of EO of a plant can be explained by factors such as: the time and place of harvest, the method of drying and extraction, the time of extraction, age, season, vegetative stage and / or the part of the plant extracted [20, 23]. From the analysis of the chemical composition of our essential oils, it appears that our oils are very rich in volatile compounds.

Diameter of essential oil inhibition zones

Diameter of zones inhibition was determined by solid medium diffusion method and the results obtained are collated in Table 2.

Table 2: Inhibition diameter zone of essential oils (mean ± sd. n = 3)

Bacteria	Gram negative						Gram positive				
Essential oils	Inhibition Diameter (mm)										
	E. co.	*E. co.* O157:H7	*P. mi.*	*P. vu.*	*P. ae.*	*S. ty.*	*S. au.*	*S. ep.*	*S. or.*	*E. fa.*	*M. lu.*
C. citratus	17,25±3.20	20±0.00	19.5±0.71	43.75±0.5	30 ± 0.00	17.5±3.51	21±0.81	25±3.20	40.5±0.71	22.5±0.71	32.25±2.06
C. giganteus	-	11±1.41	9.5±0.71	-	17.5±0.71	-	12.5±0.71	14.5±0.5	12±1.41	-	-
C. nardus	-	-	9.25±0.95	9±1.41	16.25±1.5	-	18±1.41	17.25±2.62	nd	-	10.5±0.71
C. schoenantus	9.75±0.5	10.75±0.5	-	9±1.73	20.5±0.71	14.5±0.71	16.75±1.5	20 ± 0.00	10±2.45	15.5±0.71	19.5±0.71
P. guajava	-	16.5±2.06	10.5±0.57	11±1.15	10±0.00	-	19.75±0.57	-	-	10±1.41	9.5±0.57
E. camaldulensis	-	-	20.5±0.71	11.75±1.53	10.5±0.71	-	-	-	-	-	-
C. aurantifolia	31.5±0.71	17.5±2.06	27±2.94	31.5 ± 2.94	23±2.94	21±1.41	17±1.83	16.75±1.20	13.25±1.70	21.25±0.71	11.75±0.71

E. co.: Escherichia coli, P. mi.: Proteus mirabilis, P. vu.: Proteus vulgaris, P. ae.: Pseudomonas aeruginosa, S. ty.: Salmonella typhi, S. au.: Staphylococcus aureus, S. ep.: Staphylococcus epidermidis, S. or.: Streptococcus oralis, E. fa.: Enterococcus faecalis, M. lu.: Micrococcus luteus, P. guajava: Psidium guajava, E. Camaldulensis: Eucalyptus Camaldulensis, C. aurantifolia: Citrus aurantifolia, C.: Cymbopogon, (-) = not detected

According to [44], the diameter of the zones of inhibition is depend on four activity levels: low (D <8 mm); medium (9 mm ≤ D ≤ 14 mm); strong (15mm ≤ D ≤ 19mm); very strong (D> 20 mm). The analysis of table 2 shows that the oils have inhibited at least three of the strains tested. Two oils come first with inhibitory activity on all strains: these are *C. aurantifolia* and *C. citratus.*

According to [44], *C. citratus* oil exhibits very strong activity on grams positive and at least strong activity on grams negative. *C. aurantifolia* shows a very strong activity on grams negative except *E. coli* O157:H7 a strong activity. On the positive grams, it shows at least average activity.

[10], using pure oil on whatman paper, obtained for the EO of *C. citratus*, a very strong zone of inhibition for the bacteria *E. faecalis* (34 mm) and *S. aureus* (24.3 mm), a strong zone of inhibition on *E. coli* (15.3 mm) and showed that the oil has no effect on *P. aeruginosa.* Furthermore, [45] found that the inhibitory effect of the oil increases with its concentration. Thus by varying the concentration of the oil, they showed that the bacteria *S. aureus* and *E. coli* are sensitive with of zones inhibition (14.33–29.66 mm) and (8.33–22.66 mm) respectively, but that the oil has no effect on *P. aeruginosa.* These reports are in agreement with our results, except that our *C. citrtus* oil also inhibited *P. aeruginosa* (30 mm) at 20 mg/mL. This may be due to the difference in chemical composition of the oil.

The EO of *C. schoenanthus*, inhibited the growth of ten strains tested with zones inhibition varying from 9 mm to 20.50 mm. The oil has a strong effect against gram-positive bacteria except on *S. oralis* (10 mm) and at least a medium effect on gram-negative ones. However, it has no effect on *P. mirabilis*. In the literature, [13] reported that the inhibitory effect of *C. schoenanthus* oil increases with increasing concentration. Among Gram-positive bacteria, *S. aureus* was the most sensitive strain (28-34 mm) followed by *E. faecalis* (19-25 mm). For the Gram-negative bacteria tested by these authors, best activity was observed for *P. mirabilis* (21-24 mm), *E. coli* (18-21 mm) and *E. coli* O157:H7 (17-19 mm) while *P. aeruginosa* showed weak zones of inhibition (8-11 mm). Other authors, [11], report 15 mm and 19.5 mm respectively for *E. coli* and *S. aureus*. These reported results confirm the inhibition of the same strains, but the inhibition diameters indicated remain greater than those of our oil. These authors worked with pure oil on whatman paper.

P. guajava oil inhibits 7 (seven) strains with moderate activity on gram positive and negative, except *S. aureus* (19.75 mm) and *E. coli* O157:H7 (16.5 mm) on which, it has a strong inhibitory activity.

Two of the oils studied show inhibitory activity on six strains with at least moderate areas of inhibition against grams positive and negative. Among them, that of *C. nardus* strongly inhibits three strains (*P. aeruginosa*, *S. aureus* and *S. epidermidis*) while the only strain strongly inhibited by oil of *C. giganteus* is *P. aeruginosa*. For EO of *C. giganteus*, [10] obtained a very strong zone of inhibition for the bacteria *E. faecalis* and *E. coli* (24 mm), *S. aureus* (28.3 mm) and *P. aeruginosa* (20.3 mm). This confirms inhibitory activity of EO.

Only *E. camaldulensis* oil remains without activity on gram positive bacteria tested. It nevertheless exhibits strong activity on *P. mirabilus* and moderate activity on *P. vulgaris* and *P. aeruginosa*, all of which are gram negative bacteria. In literature it has been also indicated inhibitory activities of this oil on gram positive bacteria. Indeed, [7] reported at 500 µg/mL, a zone inhibition for *P. aeruginosa* and *E. coli* (12.5 mm) and *S. typhi* (11 mm), all gram negative; but also a diameter of 10 mm for *E. faecalis*, a gram positive bacterium. Other authors, [46], for 10 µL of oil diluted at different concentrations on whatman paper, obtained a diameter for *E. coli* (10-31 mm) and *S. aureus* (10 to 26 mm) [47] were found for a concentration of 2 to 4 µL, for *E. coli* (8-11 mm) and *S. aureus* (10 to 16 mm) [8] obtained at 20 µL of pure oil on whatman paper, for *S. aureus* (21 mm) and *E. coli* (10 mm). These different results obtained in the literature could be explained by the difference in the chemical composition of EO in plants.

The spectrum of bacteria inhibited by our EO partially confirms the use of these plants by the population for infection control.

Minimum Inhibitory Concentrations (MIC)

MIC of EO are shown in Table 3. The table shows that our EO inhibits the growth of bacteria studied with MICs values ranging from 0.312 to 5 mg/mL. The lowest MIC value is obtained at 0.312 mg/mL for the EO of *C. aurantifolia* (on *E. coli*, *P. mirabilus*, *P. vulgaris* and *S. typhi*); *C. citratus* (on *E. coli* O157: H7, *P. vulgaris* and *P. aeruginosa*); *C. nardus* (on *S. aureus*) and *C. schoenantus* (on *S. aureus*, *M. luteus* and *P. aeruginosa*). The greatest MIC value is 5 mg/mL for *E. camaldulensis* (on *P. vulgaris*) and *C. giganteus* (on *P. mirabilus*) [48] proposed a classification of plant EO, on the basis of MIC results, which is as follows: strong inhibition (MIC < 0.5 mg/mL), moderate inhibition (0.6mg/mL ≤ MIC ≤ 1.5mg/mL), low inhibition (MIC > 1.6 mg/mL).

According to this classification, the oils which strongly inhibit gram positive bacteria are *C. nardus* and *C. schoenantus* respectively on *S. aureus* and *S. aureus*, *M. luteus*. On the other germs, the oils showed moderate or weak inhibition. It should be noted that the oil of *E. camaldulensis* did not inhibit any gram positive tested (Table 3).

Regarding gram negative bacteria, the oils studied inhibited at least one of the bacteria tested. The strongest inhibitions are found at the level of *C. aurantifolia* (on *E. coli*, *P. mirabilus*, *P. vulgaris* and *S. typhi*), *C. citratus* (on *E. coli* O157: H7, *P. vulgaris* and *P. aeruginosa*), *C. schoenantus* (on *P. aeruginosa*).

Table 3: Minimum Inhibitory (MIC) and Bactericidal (MBC) Concentrations, and Antibiotic power (MBC / MIC)

Bacteria		Gram negative						Gram positive				
Essential oils	**Parameters (mg/mL)**	***E. co.***	***E. co.* O157:H7**	***P. mi.***	***P. vu.***	***P. ae.***	***S. ty.***	***S. au.***	***S. ep.***	***S. or.***	***E. fa.***	***M. lu.***
C. citratus	MIC	0.625	0.312	0.625	0.312	0.312	0.625	0.625	0.625	0.625	0.625	0.625
	MBC	2.5	5	5	1.25	2.5	10	1.25	5	5	5	2.5
	MBC/MIC	4*	16	8	4*	8	16	2*	8	8	8	4*
C. giganteus	MIC	–	2.5	5	–	0.625	–	2.5	1.25	2.5	–	–
	MBC	–	10	> 10	–	2.5	–	5	5	10	–	–
	MBC/MIC	–	4*	–	–	4*	–	2*	4*	4*	–	–
C. nardus	MIC	–	–	2.5	2.5	0.625	–	0.312	1.25	–	–	2.5
	MBC	–	–	> 10	> 10	2.5	–	5	5	–	–	> 10
	MBC/MIC	–	–	–	–	4*	–	16	4*	–	–	–
C. schoenantus	MIC	2.5	2.5	–	2.5	0.312	2.5	0.312	0.625	2.5	1.25	0.312
	MBC	5	5	–	> 10	2.5	10	1.25	2.5	> 10	5	2.5
	MBC/MIC	2*	2*	–	–	8	4*	4*	4*	–	4*	8
P. guajava	MIC	–	1.25	2.5	2.5	2.5	–	0.625	–	–	2.5	2.5
	MBC	–	2.5	> 10	> 10	5	–	5	–	–	> 10	> 10
	MBC/MIC	–	2*	–	–	2*	–	8	–	–	–	–
E. camaldulensis	MIC	–	–	0.625	5	2.5	–	–	–	–	–	–
	MBC	–	–	5	> 10	> 10	–	–	–	–	–	–
	MBC/MIC	–	–	8	–	–	–	–	–	–	–	–
C. aurantifolia	MIC	0.312	0.625	0.312	0.312	0.625	0.312	0.625	0.625	2.5	0.625	0.625
	MBC	1.25	10	2.5	2.5	5	5	2.5	2.5	> 10	2.5	5
	MBC/MIC	4*	16	8	8	8	16	4*	4*	–	4*	8

MBC/MIC with * (Bactericidal effects) and without * (Bacteriostatical effects), *E. co.: Escherichia coli, P. mi.: Proteus mirabilis, P. vu.: Proteus vulgaris, P. ae.: Pseudomonas aeruginosa, S. ty.: Salmonella typhi, S. au.: Staphylococcus aureus, S. ep.: Staphylococcus epidermidis, S. or.: streptococcus oralis, E. fa.: Enterococcus faecalis, M. lu.: Micrococcus luteus, P. guajava: Psidium guajava, E. Camaldulensis: Eucalyptus Camaldulensis, C. aurantifolia: Citrus aurantifolia, C.: Cymbopogon,* (-) = not detected

Among all the oils studied, two stand out with at least weak inhibitory activity on all bacteria tested. These are oil of *C. aurantifolia* and *C. citratus*. These results confirm the work of [9, 10, 45, 49] concerning the oil of *C. citratus*, except for *P. aeruginosa* according to [45]. For *C. aurantifolia* oil, [12] found antimicrobial activity on *S. aureus* (0.00781 mg/mL) and reported that the oil is without activity on *P. aeruginosa*.

P. mirabilus was the only germ that remained insensitive to *C. schoenantus* oil, the other oils inhibited seven bacteria (*P. guajava*), six bacteria (*C. giganteus* and *C. nardus*) and three bacteria (*E. camaldulensis*).

From our study, it appears that of all the germs tested, *E. camaldulensis* showed only weak inhibition in three of the gram negative bacteria. However, in the literature, it has a strong activity on *E. coli* (0.05 mg/mL [7], 0.008 mg/mL [50]), on *S. typhi* (0.05 mg/mL [7]), on *E. faecalis* (0.025 mg/mL [7]), *P. aeruginosa* (0.2 mg/mL [7]), *S. aureus* (0.008 mg/mL [50], 0.312, 1.25 and 2.5 mg/mL respectively for Tweens 60, 40 and 20 [5]).

For *C. schoenantus* oil, our work is consistent with that of [51] (*E. coli* (0.016 mg/mL), *P. aeruginosa* (> 1 mg/mL) and *S. aureus* (0.016 mg/mL)), [6] (*S. aureus* (2.63 mg/mL) and *E. coli* (2.63 mg/mL)). Contrary to the results of our study for *C. giganteus* oil, [6] showed activity on *E. coli* (0.64 mg/mL) and [10] on *E. faecalis* (6.7 mg/mL) and *E. coli* (6.3 mg/mL). Similarly, for *C. nardus* oil, [52] obtained inhibition of *E. coli* O157:H7. These differences in results could be explained by the difference in chemical composition related to place of harvest of plants.

Minimum Bactericidal Concentrations (MBC)

It is this parameter that makes it possible to determine the bactericidal effect of the EOs studied. The minimum bactericidal concentrations (MBC) of EO are given in table 3. From the analysis of the table, it appears that our oils are bactericidal against the strains inhibited with concentrations varying between 1.25 and 10 mg/mL. The best bactericidal effect is obtained with the lowest value of the MBC (1.25 mg/mL) for the EO of *C. aurantifolia* (on *E. coli*), *C. citratus* (on *S. aureus* and *P. vulgaris*) and *C. schoenantus* (on *S. aureus*). *P. guajava* (on *P. vulgaris*), *C. aurantifolia* (on *E. coli* O157:H7), *C. citratus* (on *S. typhi*), *C. giganteus* (on *E. coli* O157:H7) and *C. schoenantus* (on *S. typhi*) a MBC of 10 mg/mL was obtained. Finally, the strains which were sensitive to the different extracts and whose MBC are greater than 10 mg/mL have not been determined.

Antibiotic power (MBC / MIC)

According to [53], the MBC/MIC ratio allows us to better appreciate the antibiotic power of essential oil. When this ratio MBC/MIC $\leq$ 4, extract is said to be bactericidal and when MBC/MIC > 4, extract is qualified as bacteriostatic. This ratio was calculated and recorded in Table 3.

The analysis of Table 3 shows that the EO studied exert bactericidal or bacteriostatic effects on the different strains inhibited. The EO of *C. schoenantus*, *C. giganteus*, *C. citratus* and *C. aurantifolia* are the most bactericidal (Table 3). This variability in the bactericidal power of essential oils is certainly due to the sensitivity of microorganisms to different chemical compounds which depend on the nature of the plant species, chemotypes and climatic conditions [54]. In addition, the spectrum of action of the antibacterial power of EO is very broad insofar as it reflects in a way the diversity of their chemical compounds [54]. We also note that the belonging of bacterial strains to gram (+) or gram (-) does not influence their sensitivity and this has already been demonstrated by [55].

Correlation between antimicrobial activity and chemical composition of essential oils

Study of the antimicrobial activity of EO is interesting for countering the ability of bacteria to develop bioresistance against the classic antibiotics used. Indeed, the diversity of these EO in chemical compound gives them a broad spectrum of antimicrobial effects.

From the analysis of the results of antimicrobial activity and chemical composition, it emerges that the EO of *E. camaldulensis*, the least oxygenated (14.9%), is the least active on the bacteria tested. The other EO, more active on the bacteria tested, are all rich in oxygenated compound (28.4% to 87.0%). In addition, with the exception of *P. guajava*, EO rich in sesquiterpene oxygenated compounds (28.4%), the other EO, richer in oxygenated monoterpene compounds (58.8% to 85.3%) are the most bactericidal. A synergy of action of the compounds present in the oils seems to be responsible for the antimicrobial and bactericidal activity of the EO studied. In the literature, [56] reported oxygenated monoterpenes to be responsible for the antimicrobial activity of several EO. Thus, to effectively deal with the ailments for which the various bacteria studied are responsible, these bactericidal EO could be used.

Cytotoxicity of essential oils

Cytotoxic activity of EOs was evaluated on Chinese Hamster CHO ovary cells and on human embryonic lung fibroblasts WI38. The results of the tests are given in Table 4 and are expressed as IC_{50}.

Analysis of this table shows that cytotoxicity varies from plant to plant. The IC_{50} values of the oils of *C. giganteus*, *C. nardus*, *C. schoenantus* and *E. camaldulensis* are greater than 50 µg/mL on both cell strains. But oils of *P. guajava*, *C. aurantifolia* and *C. citratus* show respectively values of 39.00 ± 0.8 µg/mL and 38 ± 2.00 µg/mL, 31.26 ± 3.23 µg/mL and > 50 µg/mL then 10.63 ± 0.72 µg/mL and 39.77 ± 3.31 µg/mL on CHO and WI38 respectively (Table 4).

Table 4: Cytotoxicity activity of essential oils.

Essential oils	Cytotoxicity (IC_{50}, µg/mL)	
	CHO	WI38
E. camaldulensis	>50^{e}	>50^{c}
P. guajava	39.00 ± 0.80^{d}	38.00 ± 2.00^{b}
C. aurantifolia	31.26 ± 3.23^{c}	>50c
C. citratus	10.63 ± 0.72^{b}	39.77 ± 3.31^{b}
C. giganteus	>50^{e}	>50^{c}
C. nardus	>50^{e}	>50^{c}
C. schoenantus	>50^{e}	>50^{c}
Campthotecin	0.74 ± 0.09^{a}	0.44 ± 0.12^{a}

Data in the same column followed by different letters (a,b,c,…) are statistically different by Student's t-test ($P< 0.05$).

Overall, these values remain above 30 µg/mL except for the EO of *C. citratus* on CHO (10.63 ± 0.72 µg/mL) and are at least 40 times greater than that of camptothecin, reference compound (IC_{50} = 0.74 µg/mL on CHO cells and IC_{50} = 0.44 µg/mL on WI38 cells). The EO of *C. citratus*, on the other hand, has a value at least 14 times greater than that of the reference compound for CHO. These oils are therefore less cytotoxic than camptothecin.

Furthermore, EO can be cytotoxic on animal and human cells. In the literature, [57], evaluated for EO of *C. citratus*, the cytotoxic concentration CC_{50} on representative cells of human skin (HaCaT lines (keratinocytes), SK-MEL-28 (melanocytes), MRC- 5 (fibroblasts)) and obtains values for HaCaT (CC_{50} = 92.1 µg/mL), SK-MEL-28 (CC_{50} = 47.9 µg/mL) and MRC-5 (CC_{50} = 40.5 µg/mL) with the most cytotoxic MRC-5 line. In addition, he noted that the EO of *C. citratus* is toxic for the lines HaCaT, SK-MEL28 and MRC5 cells at high concentrations, with 100% dead cells at 1 mg/mL. Likewise [59], obtained a CC_{50} concentration = 150 µL/mL and CC_{50} = 450 µL/mL respectively for *C. citratus* and *C. nardus* on the HaCaT cell line. From [35], obtained for the EO of *C. nardus* on the HepG-2 (hepatic) and MRC-5 cell lines, respectively, IC_{50} concentrations = 96.6 µg/mL and 33.1 µg/mL. [59], obtained for the EO of *P. guajava* against the Vero line of kidney epithelial cells extracted from a monkey concentration IC_{50} = 37.54 µg/mL.

But we find that our EO destroy animal cells at concentrations lower than those allowing the destruction of human cells. However, it would be desirable to carry out additional toxicity tests on *C. citratus* oil. It appears from cytotoxicity tests that our oils are not cytotoxic. This would explain the use of these plants without great risk of intoxication in traditional medicine by the population for the fight against infection.

Conclusion

The emergence of microbes has led to the uncontrolled use of antimicrobial drugs for infection control. This has generated resistance and is a public health problem around in world. The present Work, consists in determined the antibacterial and cytotoxic activity of seven essential oils. The results work show that our plants are important natural sources of antibacterial substances.

The evaluation of antimicrobial activities shows that EO of *E. camaldulensis*, the least oxygenated, is the least active on the bacteria tested. The other EO, more active on the germs tested, are all rich in oxygenated compound. A synergistic action of the compounds present in the oils seems to be responsible for the antimicrobial activity of the EO studied. The EO studied are less toxic than campthotecin, a reference compound, and therefore can be recommended for antimicrobial control.

Conflict of Interest

The authors have not declared any conflict of interest.

Contributions from Authors

Each author contributed equitably.

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