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Research Article

Chemical composition and antibacterial potential of *Cymbopogon citratus* essential oil on bacterial etiologies of diarrhea

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Abstract

Diarrheal infections caused by bacteria remain a major health threat in several areas worldwide. This situation is exacerbated by the excessive selection and dissemination of resistant etiologies, and the scarcity of holistic control technologies. The present study was conducted on the essential oil (EO), extracted from the leaves of *Cymbopogon citratus* that were harvested in the city of Douala, Cameroon. This study aimed to identify the chemical compounds in the EO and assess its antibacterial potential against bacterial isolates involved in diarrhea by two concurrent techniques. The EO was extracted by hydrodistillation, followed by chemical screening conducted by gas chromatography coupled with mass spectrometry (GC-MS). The antibacterial potential was tested by EO dilution in broth and agar, carried out on 12 isolates, of which some underwent susceptibility tests by standard disk diffusion. The extraction yield was 0.31% w/w. This oil was found to be rich in monoterpenes namely neral (41.24%), geranial (33.52%), and β-pinene (6.11%). The minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) varied from 1.54 to 40.83 mg/mL and 4.08 to 89.78 mg/mL in broth while in agar, the respective values ranged from 1.54 to 12.25 mg/mL and 6.12 to 98 mg/mL, and were found to be proportional to the inhibition zone diameters generated by agar diffusion. Overall the findings justify, at least partially, the use of *Cymbopogon citratus* essential oil in many traditional medicines.

1. Introduction

According to the WHO, acute diarrhea refers to the release of at least three loose or watery stools per day for less than 14 days [1]. This condition results from the altered physiology of the bowel, which allows an increase in the frequency of loose dejections caused by a higher outflow of water and/or electrolytes from body systems into the gut lumen [2]. Diarrheal diseases represent a major health threat worldwide, with escalating occurrence and consequences in developing countries, where conducive environmental determinants often prevail [3]. They are also recognized as the second leading cause of death among children under five years of age, with about 760,000 cases per year; 80% of whom die during the first two years of life [4]. Globally, the peak of infection occurs between the first week and the 18th month of life. On average, it is estimated that before the age of five, every child experiences three to nine episodes of diarrhea per year. According to some sources, childhood diarrhea is the fourth leading cause of mortality in children under five years of age in Cameroon, after malaria, measles, and respiratory tract infections [5–7]. Bacterial diarrhea is common in low- and middle-income countries, mainly due to limited access to drinking water and poor environmental sanitation, in connection with food

processing and storage conditions. Most common etiologies include members of the *Enterobacteriaceae* family like *Escherichia coli* (*E. coli*), *Salmonella* (most often non-typhoidal), *Shigella*, and *Campylobacter* [8].

The therapeutic caretaking currently in force relies on rehydration and zinc supplementation [8]; however, antibacterial drugs are often required for disease control. Accordingly, and most commonly, this antibiotic therapy is very often inappropriate and sometimes reported to either cause symptom aggravation, and/or represent a potent engine for the selection and dissemination of drug-resistant strains, which further undermines holistic disease control policies [9]. Combined with some side effects like disorders associated with disbalance in the residual intestinal flora, antabuse effect, photosensitivity, hepatotoxicity and headaches, the low purchasing power of the exposed populations also contributes to the overall poor outcome at the individual and community scale in low- and middle-income areas [9]. To control these caretaking breaks, phytomedicine has emerged as an alternative that deserves attention, in connection with resource availability and indigenous practices. In fact, Cameroon's flora is rich in plant species from which derivatives like essential oils are traditionally used to control human diseases [10].

Essential oils are natural fluids predominantly composed of terpenoids (mainly mono- and sesquiterpenes) and aromatic compounds derived from phenylpropane [11, 12]. These aromatic compounds can be obtained from raw plant materials through distillation or mechanical processes. Insoluble in water, they can be dissolved in other solvents such as acetone, carbon disulfide, chloroform, and methylene chloride [13, 14]. They are also known to have broad-spectrum antimicrobial potential against bacteria and fungi [15–17], and are used in food processing and the cosmetic industry for their flavor and organoleptic properties [11, 12]. In disease control, they can be used as anti-infectives, anti-inflammatories, analgesics, antitumor, and bronchial fluidifiers [18–20]. In this vein, one of the plants that are used in folk medicine is *Cymbopogon citratus* (*C. citratus*). *C. citratus* commonly known as lemongrass, belongs to the *Poaceae* family within the *Cymbopogon* genus. In traditional medicine, *C. citratus* is used to treat a wide range of disorders. According to user populations, products from leaf decoction or essential oil (EO) are used to control conditions such as gastrointestinal disorders, coughs, herpes, fever, headaches, heart disorders, sickle cell anemia, flatulence, vomiting, dyspepsia, jaundice, insomnia, snake bites, whooping cough, elephantiasis, and depression [21, 22]. In Côte d'Ivoire, a decoction from the dried leaves of this plant is orally administered to manage high blood pressure. Mauritians and Thais use the decoction as bath water to relieve bone and joint pain and to reduce or stop postpartum hemorrhage and diarrhea [22]. In the Caribbean, decoctions of *C. citratus* and *Momordia charantia* (*Curcubitaceae*) leaves are used for their hypotensive effects [22]. Ethnobotanical surveys have revealed that infusions or decoctions of *C. citratus* leaves, either alone or in combination with *Cassia occidentalis* and *Citrus aurantifolia*, are used in Côte d'Ivoire, Nigeria, and Ghana to control malaria, especially during pregnancy [22]. Two methods (dilution in broth and agar) are concurrently used in laboratories to test the antibacterial potential of plant derivatives, without clear choice justification.

The present study aimed to substantiate the antibacterial potential of the EO extracted from *C. citratus*, by performing two *in vitro* tests (in broth and agar) and identifying several secondary metabolites by chromatography/mass spectrometry (GC-MS) and anticipating their role in the overall antibacterial potential. The findings will contribute to achieving the United Nations N° 1st, 3rd, 4th, and 10th WHO objectives for the 2030 Sustainable Development Goals on poverty alleviation, good health and well-being, quality education, and reduced inequality, respectively.

2. Materials and methods

2.1. Plant material and extraction of the essential oil

The dried leaves of *C. citratus* were harvested from Douala (Littoral region of Cameroon) in December 2021, and identified at the National Herbarium of Cameroon under reference N° 18628 HNC. The EO was extracted by hydrodistillation [13] using a Clevenger-type apparatus at the Laboratory of

Chemistry of the Université des Montagnes. The chopped and weighed plant material (10 kg) was mixed with 8 liters of water in a flask. The resulting mixture was heated to ebullition for 4 h. The vapors containing volatile components were allowed to pass through a refrigerant column, where it condensed, and were collected in a separating funnel. In the funnel, water and oil phases were clearly separated based on their densities and could be extracted. The extracted light-yellow EO was then dried with anhydrous sodium sulfate, weighted and stored at 5°C in dark vials until use.

2.2. Chemical composition of the extracted essential oil

The EO obtained was analyzed by gas chromatography (GC) and gas chromatography coupled with mass spectrometry (GC/MS).

2.2.1. Gas chromatography coupled with flame ionization detector (GC/FID)

The EO was analyzed on a Varian CP-3380 GC with flame ionization detector fitted with a fused silica capillary column (30 m × 0.25 mm coated with DB5, film thickness 0.25 µm); temperature program 50-200 °C at 5 °C/min, injector temperature 200 °C, detector temperature 200 °C, carrier gas nitrogen (N2), 1 mL/min. The linear retention indices of the components were determined in connection with the retention times of a series of n-alkanes, while the percentage compositions were obtained from electronic integration measurements without considering the relative response factors.

2.2.2. Gas chromatography coupled with mass spectrometry (GC/MS) analysis

GC/MS analyses were performed using a Hewlett-Packard apparatus equipped with an HP1 fused silica column (30 m × 0.25 mm, film thickness 0.25 µm) interfaced with a quadrupole detector (GC-quadrupole MS system, model 5970). The column temperature was programmed from 70-200 °C at 10 °C/min; the injector temperature was 200 °C. Helium was used as the carrier gas at a flow rate of 0.6 mL/min. Other characteristics include the mass spectrometer operated at 70 eV, and the injection volume (0.1 µL) of an EO solution diluted to 10% in hexane. The identification of individual compounds was based on a comparison of their relative retention times and their mass spectra of peaks with those obtained from authentic samples in libraries and published data [23-25].

2.3. Evaluation of the antibacterial potential

2.3.1. Preparation of culture media, isolation and identification of bacteria

All microbiological investigations were conducted at the Laboratory of Microbiology, Université des Montagnes Teaching Hospital (West-Cameroon). They were carried out on bacterial isolates recovered from routine clinical specimens from June 2021 to May 2022. For all bacteriological procedures, culture media (Bio-Rad(R)) was prepared as recommended by the manufacturer. They included Mueller Hinton agar, Muller Hinton Broth, Nutrient agar, Simmons citrate agar, Mannitol-mobility agar, and Kligler-Hajna agar. Bacterial isolation and identification were performed according to standard procedures [26]. Briefly, microscopy characterization (Gram stains) and biochemical identification tests were conducted for key parameters. These included tests for oxidase, carbohydrates (mannitol, lactose, and glucose) fermentation, motility, urea hydrolysis, indole and tryptophanase production, citrate metabolism tests. *Escherichia coli* ATCC 25922 was used as a reference bacterial strain for quality control throughout the process.

Upon completion of identification, twelve isolates were subjected to the tests, namely *Citrobacter freundii, Klebsiella spp*, *Escherichia coli, Enterobacter cloaceae, Klebsiella oxytoca, Enterobacter aerogenes, Serratia ficaria, Providencia rettgeri, Proteus spp., Enterobacter agglomerans, Serratia marcescens* and *Vibrio spp*. Out of these, *C. freundii, Klebsiella oxytoca, Escherichia coli, Vibrio spp., Proteus spp*., and *Serratia marcescens* were underwent conventional susceptibility testing by standard disk diffusion [27].

2.3.2. Investigating the antibacterial potential of the essential oil on isolates

This step was conducted with 24 h pure cultures of the subjected bacterial populations grown aerobically at 37°C on nutrient agar. The inhibition diameters were assessed according to standard procedures for disk diffusion with slight modifications to test the inherent EO activity. Briefly, for this purpose, 5 µL of the high-concentration oil solution was allowed to diffuse from a 6-mm Whatman N° 2 paper disk firmly, but

delicately adjusted to the subjected bacterial lawn on Muller Hinton agar in a 90 mm-diameter Petri dish. The antibacterial activity was indicated by an inhibition area that developed around the paper disk after 24 h incubation at 37°C. This qualitative study was a prerequisite for the quantitative tests, which determined the minimal inhibitory and minimal bactericidal concentrations, as described in the previous studies [15, 17].

2.3.3. Preparation of the essential oil original solution and the bacterial inoculum

The EO (quantified in milligrams) was diluted in a mixture of 95% sterile distilled water and 5% Tween 80 (1/10 ratio). For the bacterial inoculum, the whole process was conducted with 24 h fresh bacterial pure cultures grown on nutrient agar. For this purpose, a bacterial suspension adjusted to 0.5 turbidity scale of the McFarland standard was prepared in 0.9% physiological saline and then, readjusted to the final opacity recommended for susceptibility tests by agar diffusion on Mueller Hinton. All test procedures and interpretations were performed according to the standard reference guidelines provided by the "Comité de l'Antibiogramme de la Société Française de Microbiologie" [27].

2.3.4. Determination of the Minimal Inhibitory and Bactericidal Concentrations

2.3.4.1. Assay in liquid medium

This was conducted with the broth dilution method. For this purpose, 2000 µL of Mueller Hinton broth was dispensed in fourteen (14) glass test-tubes (11 test and 3 control test-tubes). These preparations were then sterilized at 121°C for 15 min. After sterilization, the test tubes containing the broth were kept in a water bath at 37°C for 15 min. Then, 2000 µL of the mother solution (original solution) containing oil was dispensed into the first tube of the dilution series. Thereafter, a 2 step serial dilution was performed to eventually produce concentrations ranging from 98 mg/mL to 0.1 mg/mL of the EO. To each of these preparations in test tubes, 15 µL of the bacterial 0.5 McFarland inoculum was aseptically added. All preparations were then incubated aerobically for 24 h at 37°C. After incubation, turbidity was assessed visually. The MIC of each extract was determined using the first tube containing the lowest concentration of EO in which no turbidity was observed. To assess the minimal bactericidal concentrations, 0.5 µL of the homogenized suspension in tubes with no turbidity was streaked on a Muller Hinton agar plate. After overnight aerobic incubation at 37°C, the MBC was recorded from the tube containing the lowest EO concentration for which no physical bacterial growth was observed. This procedure was conducted in triplicate.

2.3.4.2. Assay in solid medium

This assay in solid medium is an adaptation of the agar dilution protocol. It was used in the present study as a simplified alternative to the tests on agar plates which require more resources to be conducted on a plate containing a specific concentration of anti-infective agents. In addition, this procedure relies on visible colony growth that can be observed on the agar, regardless of the color of the extract acknowledging that this color could greatly influence the reading accuracy in liquid medium. Except for the fact that the medium used was Mueller Hinton agar, the procedure used to determine the minimal inhibitory concentration was the same as that observed in liquid medium, as described above. Briefly, the protocol used for serial dilution was the same as that used for the assays in liquid medium, including 14 tubes for the tests and 3 tubes for the controls (positive, negative, and mother solution sterility). Once the preparation in the test tubes was completed, the tubes were removed from the water bath and allowed to solidify at room temperature, and stabilized to make a base and a slope over this base. Into each of the solidified media, 5 µL of the 0.5 McFarland bacterial inoculum was aseptically lawned on the slope, prior to incubation which was eventually conducted aerobically at 37°C for 24 h. Upon completion of incubation, the MIC was determined as the lowest EO concentration in the range at which no visible bacterial growth (colony) was recorded on the slope. To determine the minimal bactericidal concentrations, 500 µL of Muller Hinton broth was aseptically dispensed in all preparations from with no such growth was recorded in the MIC test (that is, the ones from which the MIC was determined and the concentrations above). These preparations were also aerobically incubated for 24 h at 37°C. Upon

completion, the minimal bactericidal concentrations were identified as the lowest concentration at which no turbid feature was recorded. All tests were performed in triplicate to ensure reproducibility. For all assays (in broth and agar), reading were performed blindly and separately by 15 laboratory technologists. The result was approved when 12 out of 15 (80%) reported the same value.

2.3.5 Inhibition zone diameters

The recorded concentration values of the EO were reproduced for MIC and MBC. From each solution at MIC and MBC, 10 µL was dispensed on a sterile 6-mm Whatman paper N°2 disk previously firmly adjusted on Muller Hinton agar in Petri on which the isolate subjected was pated. Then the preparation was incubated aerobically at 37°C overnight. After incubation, the inhibition zone diameter surrounding the paper disk was measured. Each assay was conducted seven times. The average values with standard deviations obtained for the seven tests were expressed in millimeter. This procedure was conducted for all MICs and MBCs values recorded during the assays in liquid and solid media.

2.3.6. Evaluation of CMB /CMI ratios

All MBC/MIC values ≥ 4 reflected the bacteriostatic potential of the EO. A value of less than 4, reflected their bactericidal potential whereas a value of 1, indicated the absolute bactericidal potential.

3. Results

3.1. Extraction yield

Table 1 displays the extraction yields recorded from the fresh leaves of *C. citratus*.

Table 1. Essential oil extraction yield.

Samples	Yields (%)
Fresh leaf weight (10 kg) Essential oil weight (31.25 g)	**0.31**

3.2. Identification of compounds using GC/MS

The GC/MS analysis of the *C. citratus* leaf EO is shown in Fig. 1 and Table 2.

According to the GC/MS analysis, a total of 16 volatile compounds were identified, including hydrocarbon monoterpenes, oxygenated monoterpenes, alcohols and ketones. Further related details are summarized in Table 2.

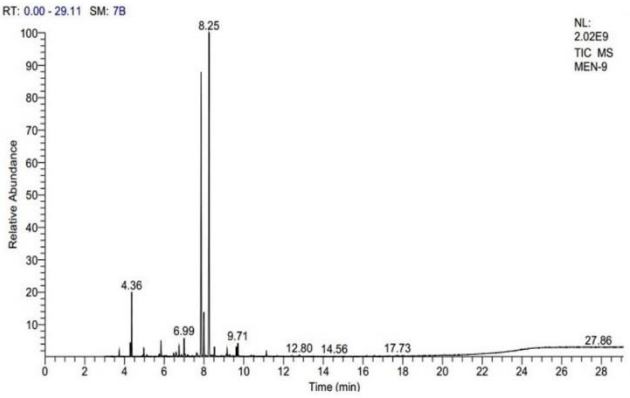


Figure 1. Representative GC-MS chromatogram of *C. citratus* essential oil.

The overall picture indicates the relative concentration of each constituent, classified according to the peak area percentage. Based on comparative qualitative analysis, the predominant constituents were identified as neral (41.24%), geranial (33.52%), and β-pinene (6.11%).

3.3. Antibacterial potential of the essential oil

3.3.1. Antibacterial assays conducted in liquid medium

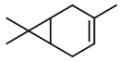
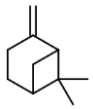
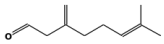
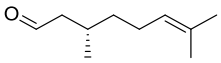
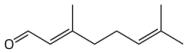
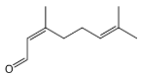
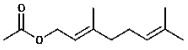
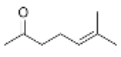
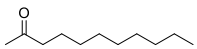
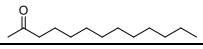
The summarized information recorded during the tests was reorganized and is shown in Table 3. It MIC values ranged from 1.54 to 40.83 mg/mL, with the lowest concentration recorded for *Escherichia coli* and the highest for *Proteus spp.* The MBCs, ranged from 4.08 mg/mL for *Enterobacter cloaceae* to 89.78 mg/mL for *Serratia marcescens.* Substantial differences were also observed between *Enterobacter cloaceae* (MBC 4.08 mg/mL) and *Enterobacter agglomerans* (MBC 65.33 mg/mL), *Klebsiella spp.* (20.41 mg/mL) and *Klebsiella oxytoca* (4.08 mg/mL) as well as between *Serratia marcescens* (20. 41 mg/mL) and *Serratia ficaria* (5.1 mg/mL). In addition, the bactericidal potential of the EO was recorded for 38% of the subjected bacterial isolates, while the MBCs were not recorded for 15% of isolates within the range of dilutions used.

3.3.2. Antibacterial assays conducted in solid medium

These studies on the antibacterial potential of EO yielded a range of results in connection with the inhibition of bacterial growth. The key pieces of information recorded for MICs, MBCs, and MBC/MIC are summarized in Table 4.

The MIC values globally ranged from 1.54 mg/mL to 12.25 mg/mL, with the lowest concentration recorded

Table 2. Identified compounds in the essential oil of *C. citratus.*

RT (min)	Identified compounds	MW (g/mol)	Chemical structure	Peak area (%)
	Hydrocarbon monoterpenes (MTH)			***6.70***
3.73	3-Carene	136.234		0.59
4.36	β-pinene	136.234		6.11
	Oxygenated monoterpenes (MTO)			***88.34***
4.97	Eucalyptol	154.249		0.82
5.83	Linalool	154.249		1.70
6.47	6-octenal,7-methyl-3-methylène	152.233		0.51
6.59	6-octenal, 3,7-dimethyl-, (S)	154.253		0.74
6.99	Isoneral	152.233		2.16
7.86	Geranial	152.233		33.52
7.99	Geraniol	154.249		5.12
8.26	Neral	152.233		41.24
9.71	Geranyl acetate	196.286		1.38
	Alcohols			***2.00***
9.16	5-Isopropenyl-2-methyl-7-oxabicyclol(4,1,0)heptan-2-ol	168.233		0.95
9.63	(2,2,6Trimethylbicyclo(4,1,0)hept- 1-yl)-methanol	168.276		1.05
	Ketones			***2.56***
4.29	6-methyl-5-hepten-2-one	126.199		0,93
8.52	Undecanone-2	170.296		0.98
11.14	2-tridecanone	198.350		0.65

RT = Retention time, MW = Molecular weight.

for *Escherichia coli* and *Klebsiella oxytoca*; and the highest for *Klebsiella spp.,* and *Providencia rettgeri.* For the MBCs, the values ranged from 6.12 mg/mL (for *Citrobacter freundii* and *Klebsiella oxytoca spp.)* to 98 mg/mL (for *Serratia ficaria)*. The bactericidal potential was observed in 46% of the isolates; with no case of absolute bactericidal activity.

3.3.3. Inhibition zone diameters by disk diffusion for the MICs and MBCs values recorded when the tests were conducted in liquid and solid media

When the paper disks adjusted on bacterial culture (lawned on Mueller Hinton in Petri dishes) were inoculated with the EO at the MICs and MBCs values, the related inhibition diameters and the inhibition

Table 3. MIC, MBC, and MBC/MIC of the essential oil in liquid medium.

Bacterial isolates	MIC (mg/mL)	MBC (mg/mL)	R	Effects
Citrobacter freundii	4.08 ± 1.76	6.12 ± 0	1.5	Bactericidal
Klebsiella spp.	20.41 ± 7.07	32.66 ± 14.14	1.6	Bactericidal
Escherichia coli	1.54 ± 0.88	12.25 ± 10.2	7.95	Bacteriostatic
Enterobacter cloaceae	2.55 ± 0.88	4.08 ± 1.76	1.6	Bactericidal
Klebsiella oxytoca	4.08 ± 1.76	20.5 ± 20.41	5.02	Bacteriostatic
Enterobacter aerogenes	2.55 ± 0.88	4.08 ± 1.76	1.6	Bactericidal
Serratia ficaria	5.1 ± 1.76	-	-	-
Providencia rettgeri	16.5 ± 6.92	75.33 ± 28.29	4.70	Bacteriostatic
Vibrio spp.	5.01 ± 1.76	32.66 ± 14.14	6.51	Bacteriostatic
Proteus spp.	40.83 ± 14.14	-	-	-
Serratia marcescens	20.41 ± 7.07	89.78 ± 28.29	4.39	Bacteriostatic
Enterobacter agglomerans	5.1 ± 1.76	65.33 ± 28.29	12	Bacteriostatic

R: Ratio = MBC/MIC

Table 4. MIC, MBC, and MBC/MIC of the essential oil in solid medium.

Bacterial isolates	MI C (mg/mL)	MBC (mg/mL)	R	Effects
Citrobacter freundii	3.06	6.12	2	Bactericidal
Klebsiella spp.	12.25	24.5	2	Bactericidal
Escherichia coli	1.54	12.25	7.95	Bacteriostatic
Enterobacter cloaceae	12.25	24.5	2	Bactericidal
Klebsiella oxytoca	1.54	6.12	3.97	Bactericidal
Enterobacter aerogenes	3.06	24.5	8	Bacteriostatic
Serratia ficaria	3.06	98	32	Bacteriostatic
Providencia rettgeri	12.25	24.5	2	Bactericidal
Vibrio spp.	6.12	49	8	Bacteriostatic
Proteus spp.	3.06	49	16.01	Bacteriostatic
Serratia marcescens	6.12	49	8	Bacteriostatic
Enterobacter agglomerans	6.12	49	8	Bacteriostatic

R: Ratio = MBC/MIC

diameters recorded with amoxicillin and levofloxacin disks are summarized and presented in Table 5 and 6.

Key related pieces of information reveal more or less large growth inhibition zones at varied MICs and MBCs. Except for *Enterobacter agglomerans* the values documented at the MBCs were larger than those observed with conventional antibiotics. Further insights indicated that the diameters were larger with levofloxacin than with amoxicillin. In addition, the diameter was smallest with *Enterobacter agglomerans.* Overall, a proportional relationship was observed between the essential oil concentration and the inhibition zone diameter. A similar trend was observed when the tests were conducted in a solid medium (Table 6).

In the overall presentation, the MICs values in liquid medium were observed similarly in 85% of cases compared to the MBCs, 90% by laboratory technologists who were called upon to read the test results. For the assays in solid medium, a 100% rate was obtained for the respective rates.

3.4. Susceptibility test on a few diarrheal disease etiologies

Susceptibility tests with conventional antibacterial agents on six etiologies of gastrointestinal disorders revealed an isolate-depended profile. Brought together, the finding are summarized in Table 7. Overall screening revealed 58% of resistance, with the higher rates observed with third generation cephalosporins (CFM, CTX, CAZ, and CRO) and higher effectiveness with aminoglycosides (GN) and fluoroquinolones (LEV and OFX).

Table 5. Inhibition zone diameters on agar plates (mm) with the values recorded for the MICs and MBCs when the tests were conducted in liquid medium compared to antibiotics.

Bacterial isolates	Essential oil		Antibiotics	
	Φ /MIC	Φ /MBC	AMX (30 µg)	LEV (5 µg)
Citrobacter freundii	15 ± 0.23	30 ± 0.23	18	23
Klebsiella spp.	20 ± 0.47	35 ± 0.47	06	29
Escherichia coli	15 ± 0.23	30 ± 0.47	26	10
Enterobacter cloaceae	26 ± 0.23	35 ± 0.23	18	30
Klebsiella oxytoca	40 ± 0.47	45 ± 0.47	14	25
Enterobacter aerogenes	26 ± 0.47	35 ± 0.23	13	30
Serratia ficaria	16 ± 0.47	40 ± 0.47	13	25
Providencia rettgeri	35 ± 0.47	40 ± 0.23	15	35
Vibrio spp.	12 ± 0.47	24 ± 1.63	11	10
Proteus spp.	20 ± 0.23	28 ± 0.81	30	21
Serratia marcescens	12 ± 0.81	24 ± 1.24	33	24
Enterobacter agglomerans	10 ± 0.62	13 ± 0.81	12	29

Φ/MIC: inhibition zone diameter recoded with minimal inhibitory concentrations of the essential oil; Φ/MBC: inhibition zone diameter recoded with minimal bactericidal concentrations of the essential oil; AMX: Amoxicillin; LEV: Levofloxacin.

Table 6. Inhibition zone diameters on agar plates (mm) with the values recorded for the MICs and MBCs when the tests were conducted in solid medium compared to antibiotics.

Bacterial isolates	Essential oil		Antibiotics	
	Φ/MIC	Φ/MBC	AMX (30 µg)	LEV (5 µg)
Citrobacter freundii	20 ± 1.24	35 ± 0.47	18	23
Klebsiella spp.	10 ± 0.47	35 ± 0.23	06	29
Escherichia coli	20 ± 0.47	35 ± 1.24	26	10
Enterobacter cloaceae	30 ± 0.23	35 ± 1.63	18	30
Klebsiella oxytoca	40 ± 0.47	45 ± 0.23	14	25
Enterobacter aerogenes	30 ± 0.47	35 ± 0.47	13	30
Serratia ficaria	10 ± 0.47	18 ± 0.23	13	25
Providencia rettgeri	10 ± 0.47	40 ± 1.24	15	35
Vibrio spp.	6 ± 0.47	28 ± 0.81	11	10
Proteus spp.	20 ± 0.23	28 ± 1.24	30	21
Serratia marcescens	10 ± 0.81	18 ± 1.24	33	24
Enterobacter agglomerans	9 ± .062	11 ± 0.81	12	29

Φ/MIC: inhibition zone diameter recoded with minimal inhibitory concentrations of the essential oil; Φ/MBC: inhibition zone diameter recoded with minimal bactericidal concentrations of the essential oil; AMX: Amoxicillin; LEV: Levofloxacin.

Table 7. Susceptibility profiles for key diarrheal etiologies.

Isolates	Antibiotics										
	CPR	OFX	LEV	CFM	CTX	AM	MET	GN	AMC	CAZ	CRO
C. freundii	S	I	R	R	R	R	S	S	R	R	R
Klebsiella spp.	S	S	R	R	R	R	S	S	R	R	S
E. coli	S	S	R	R	R	S	S	S	S	R	R
Vibrio spp.	R	R	S	R	R	R	R	S	R	I	R
Proteus spp.	I	R	S	R	R	S	R	S	S	R	R
Serratia marcescens	R	S	S	R	R	S	R	S	S	R	R

CPR: Ciprofloxacin, OFX: Ofloxacin, LEV: Levofloxacin, CFM: Cefixime, CTX: Cefotaxime, AM: Amoxicillin, MET: Metronidazole, GN: Gentamicin, AMC: Clavulanic Acid/Amoxicillin, CAZ: Ceftazidime, CRO: Ceftriaxone, S: susceptible, I: intermediate, R: resistant

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4. Discussion

The present study on the chemical composition and antibacterial potential of the EO extracted from *C. citratus* leaves against bacteria responsible for diarrhea revealed a few pieces of information in connection with the investigation.

The hydrodistillation yield was 0.31% (w/w). This value is almost half of the one reported by Venzon et al. in 2018 (0.7%), and is very low compared to the 4.31% reported by Kpatinvoh et al. [28, 29]. While resisting a clear understanding, this significant difference could (at least partially) be justified by geographical, climatic, and pedological variables that have yet to be fully highlighted. In fact, the initial work was carried out on plants harvested in southern Benin, whereas those used in the present study were harvested in the coastal area of Cameroon. Additionally, anthropic parameters and/or the extraction technique could further be pointed out [21].

GC/MS analysis of the EO revealed 16 compounds, predominantly oxygenated monoterpenes, namely neral (41.24%) and geranial (33.52%). These findings are similar to those reported in Benin (neral, 33% and geranial, 41.3%) [30]; but different from those observed in Brazzaville-Congo (neral and geranial, 32.94% and 51.99%, respectively) [31]. Moreover, some compounds identified in the present study such as β-pinene (6.11%) and isoneral (2.16%) have not been reported previously by the previous authors. In addition, the myrcene (hydrocarbon monoterpene) detected in the EO from Benin was not identified in the present investigation. Similar to the yields, the differences in the EO composition could be attributed to biotic and abiotic parameters that affect plant growth and fitness such as climate, altitude, soil type, agricultural practices, plant developmental stage, and harvesting time [32]. Furthermore, it is worth noting that citral (geranial and neral) is characteristic of the EO of *C. citratus*, regardless of its geographical origin [33]. At first glance, the antibacterial potential of the EO, the MIC values in liquid medium varied between 1.54 and 40.83 mg/mL. The lowest concentration was observed in *Escherichia coli,* and the highest was observed in *Proteus spp.* The 12.5 mg/mL reported by Katawa et al. in 2018 [34] was within the present range, similar to the bactericidal concentration. This bactericidal activity is linked to the high content of monoterpenes such as neral, geranial and β-pinene, in the EO. Aldehydes are known to disrupt the vital bacterial cell functions by acting on cellular DNA and enzymatic proteins, resulting in cell death [35]. In *Escherichia coli*, citral is known to disrupt normal physiology by increasing the permeability of the cellular membrane [36–38]. These effects could reasonably justify the bactericidal effect observed in susceptible isolates in the present investigation. However, and acknowledging the universal feature of the cell plasma membrane, additional variables are necessary to fully understand why some isolates are more susceptible than the others. The large differences, particularly observed among isolates belonging to the same genus, are consistent with these alleged partial conclusions that do not, rule out species characteristics likely to influence the degree of inhibition. However other parameters such as the inhibition diameters at the MICs and MBCs, emerge as reliable variables useful for anticipating the broad spectrum of action expressed by the EO, in line with the varieties of uses that are made of the plant extracts from one community to the other in folk medicine and the range of disorders they allegedly control. Therefore, the activity of the EO from this plant on bacterial etiologies of diarrhea can be understood.

The concentrations recorded in solid medium (agar) ranged from 1.54 to 12.25 mg/mL, which is very low compared to those obtained in liquid medium (broth) for the same isolates. The lowest concentration was observed in *Escherichia coli* and *Klebsiella oxytoca spp.*, and the highest in *Citrobacter freundii* and *Serratia ficaria*. The lower values obtained in solid medium are consistent with the conclusion that the growth environment therein is likely less conducive to bacterial survival, unlike the broth. In addition, the MIC observed for *Citrobacter freundii* was lower than the that for *Klebsiella spp*. The higher value with *Klebsiella* could be related to the bacterial capsule, which represents an additional penetration barrier to the active components of the EO expected to act on the DNA and enzymes, as discussed above.

Overall, data analysis revealed high resistance rates to the routine conventional antibiotics used (58%), likely in connection with the common and inappropriate

utilization of selective agents by the populations. The resistance rates reflect the higher likelihood of therapeutic failure in cases of gastrointestinal disorders involving these organisms. These conclusions typically rely on antibiotics that are more affected by resistance phenotypes (β-lactams), which are also the most commonly used in routine therapeutic practices. Furthermore, beyond the gastrointestinal tract, most of these bacteria could evolve as opportunistic etiologies of several human conditions such as wound and deep abscess infections which could result in septicemia [39]. Once proven, from this research and data from previous studies, future challenges reside in framing reliable paths through the sustainable use of the EO under study for the welfare of the needy population in resource-limited areas.

This requires additional investigations on the toxicity and availability of the raw material for large-scale production, in connection with suitable environmental conditions for optimal *C. citratus* plant growth. Furthermore, initiatives to produce standards that are necessary for more accurate *in vitro* projections represent pressing scientific challenges that must be addressed to establish this EO as a reliable alternative to conventional drugs. This will reduce the speed of resistance selection in microorganisms and the morbidity and mortality likelihood in vulnerable human populations. Primary indices can be drawn from the test results recorded in broth and on agar, as well as the inhibition diameters at the MICs and MBCs concentrations that are frankly expressive. Although larger than those recorded with conventional antibiotic disks in some cases, the values of inhibition diameters at the MICs and MBCs do not allow ruling out that the EO is more potent than amoxicillin and levofloxacin. Further related investigations are required to draw specific conclusions. The high resistance rate (58%) represents a threat to the control of infectious diseases caused by these bacteria, which (as developed above) currently emerge as etiologies of several diseases in humans. Therefore, disseminating related knowledge would be a special asset for better health in all areas where the properties of this plant EO could be exploited. The data from the present investigation also highlight the increased reliability of MICs and MBCs in solid medium. In fact, beyond the fact that the solid medium is closer to the standard agar dilution susceptibility test, it could be reliably used for aerobes and anaerobes.

5. Conclusions

The extraction yield of the essential oil was 0.31%, and it was composed predominantly of neral (41.24%), and geranial (33.52%). The MICs, MBCs and MBC/MIC ratios fluctuated from one isolate to another, although within acceptable ranges. All related values were lower in solid medium, where reading accuracy appeared to be the best. Although preliminary, the overall findings indicate that this oil could be considered as an alternative care-taking option in the contexts of resource limitation to suit the 2030 WHO sustainable development goals, if other variables such as those in connection with toxicity and standards, are proven suitable.

Disclaimer (artificial intelligence)

Author(s) hereby state that no generative AI tools such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators were utilized in the preparation or editing of this manuscript.

Authors' contributions

Conceptualization, V.R.T.S. and P.R.F.K; Methodology, V.R.T.S., M.Y.K.P., P.Y.M. and P.R.F.K; Essential oils extractions, M.Y.K.P. Chemical analysis, V.R.T.S., M.Y.K.P. and P.Y.M; Writing – original draft preparation, M.Y.K.P. and P.Y.M; Writing – Review & Editing, V.R.T.S. and P.R.F.K.

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Conflicts of interest

The authors declare no conflict of interest.

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