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Chemical composition and antibacterial activity of essential oils from four aromatic and medicinal plants from Niger

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Abstract
In Niger, infectious diseases are à public health concern. Medecinal plants are used traditionally for the treatment of these diseases. Four plants were selected base on their traditionnal uses. These plants are *Annona senegalensis* Pers (Annonaceae); *Cleome viscosa* L (Capparaceae); *Cymbopogon schoenanthus* Spreng (poaceae) and *Eucalyptus camaldulensis* Dehnh (myrtaceae). This study aims to determine the chemical composition of the essential oils of four plants and to evaluate their antibacterial activity. The essential oils were extracted by hydrodistillation. The chemical composition was determined by gas chromatography and mass spectrometry (GC-MS). The antibacterial activity of the essential oils was screened against pathogenic bacteria using microdilution method. *Annona senegalensis* and *Cleome viscosa* are rich in sesquiterpene, while *Cympobogon schoenanthus* and *Eucalyptus camaldulensis* are rich in monoterpene. The results of the antibacterial activity in Minimal Inhibitory Concentration (MIC) are ranging from 125-2000 µg/mL. *Annona senegalensis* showed the best activity with MIC at 125 µg/mL against four bacteria. These results show that these plants are potential sources of natural antibacterial substances that can be used to treat bacterial infections.

Keywords: Essential oils, antibacterial activity, Niger.

Introduction
In the 21st century, infectious diseases continue to wreak havoc on the human population, accounting for around half of all mortality rates in tropical countries. These alarming statistics bear witness to their devastating nature. Unfortunately, the global spread of multi-resistant bacteria has greatly reduced the effectiveness of antibacterial agents, increasing the number of therapeutic failures [1]. Moreover, infectious diseases are responsible for around 70% of child deaths in developing countries and more than a third of these deaths occur in newborns [2].
Antimicrobial resistance (AMR) has been named by the World Health Organization (WHO) as one of the three most important public health threats of the 21st century and it causes persistent infections that kill millions of persons every year with enormous demands on medical and social resources [3]. A WHO Interagency Coordination Group report on Antimicrobial Resistance (AMR) estimates that 700,000 people die each year. This number is projected to rise to 10 million by 2050 if nothing is done to stop the Antimicrobial Resistance menace [4, 5].
In Niger, infectious diseases are among the most commonly reported illnesses and are one of the biggest causes of death. According to WHO, plants are still the main resource for 80% of people in developing countries [6]. Medicinal and aromatic plants also provide an arsenal of chemical products that could be used by humans to prevent microbial invasions [7]. Their investigation is therefore a promising source for the discovery of new molecules. Niger, due to its geographical position, has a rich and varied flora. The available plant species can contain compounds with potential antimicrobial activities [8]. In Niger, some studies have reported the antibacterial activity of extracts from medicinal plants [9-12]. There are few works on the antibacterial activity of essential oils. Many aromatic and medicinal plants, herbs, and species have been proposed as very important sources of natural products with antimicrobial activity as an alternative to synthetic drugs to treat infections caused by bacteria. Annonaceae, Capparaceae, Poaceae, and Myrtaceae are some plant families used in Niger for the treatment of infectious diseases. The selected plants for the present study had indications for the treatment of infectious diseases caused by bacteria, fungi, and parasites.

They are also used as insecticidal. Ethnobotanical surveys have shown that these plants are traditionally used to treat bacterial and fungal infections [13-15]. The leaves, stems and the whole plant of *Cymbopogon schoenanthus* are used in various treatment including constipation, jaundice, hemorrhoids and infectious diseases [14]. The leaves of *E. camaldulensis* are used to treat cold, bronchitis, anemia and sickle cell diseases [14]. The aim of the present study, is to evaluate the antibacterial activity of the essential oils from four aromatic plants selected from Niger's traditionnal pharmacopiea and to determine their chemical composition.

Materials and Methods

Plants Material

The plant material are the leaves of *A. senegalensis* (leaves); *C. viscosa* (stems, leaves, flowers); *C. schoenanthus* (stems, leaves, inflorescences), and *E. camaldulensis* (leaves). These samples were identified at the Garba Mounkaila laboratory in the biology department of the Abdou Moumouni University in Niamey. The samples were dried at room temperature for five (5) days and then stored away from dust and humidity.

Methods

Extraction of essential oils

The essential oils were extracted from the plant material by hydrodistillation using a Clevenger-type apparatus. 200 g of dry plant material (*A. senegalensis*: Leaves; *C. viscosa*: Stems, leaves, and flowers; *C. schoenanthus*: Stems, leaves, and inflorescences; *E. camaldulensis*: Leaves) were coarsely cut up and placed in a 4 L flask. Distilled water (2000 mL) was added to the contents of the flask. The flask was fitted with a Clevenger-type apparatus. After starting the refrigeration, the contents of the flask were brought to the boil using a flask heater. On boiling, the azeotropic mixture of water vapor and essential oil is condensed in the condenser. Distillation takes 4 hours. At the end of the distillation, the essential oil is separated from the water by decantation and the distillation water is extracted with diethyl ether (10 mL x 3) in a separating funnel to recover the remaining essential oil.

Analysis and identification of essential oil constituents

The identification of the constituents of the essential oil obtained and determination of its relative centesimal composition were carried out by gas chromatography (GC) using a Hewlett Packard 5890 SERIES II chromatograph equipped with a flame ionisation detector (GC-FID) and by gas chromatography coupled with mass spectrometry (GC-MS).

The GC-FID analyses were carried out using a Hewlett-Packard 5890 series II chromatograph, equipped with an apolar capillary column: 50 m x 0.22 mm; film thickness: 1 µm, BPX-5 (polysilphelinene-siloxane, SGE). The oven temperature was programmed from 50°C to 150°C (3°C/min), and from 150°C to 240°C (5°C/min), and then maintained at isothermal temperature (5 min). The injector and detector temperatures were 280°C and 300°C respectively. The essential oil samples were injected using a split/splitless mode (10:1) and helium was used as the carrier gas (flow rate 1 mL/min). The centesimal composition was calculated based on the GC peak area of each compenent, without using correction factors. A volume of 0.2 µL of essential oil (dissolved in hexane as 5/100 v/v) was injected manually. GC-MS analyses were performed using a Hewlett Packard 5890 SERIE II gas chromatograph coupled to a Hewlett Packard 5971 SERIES mass spectrometer. The capillary column (SGE, BPX - 5 (50 m x 0.22 mm; film thickness: 1 µm) was connected to the mass spectrometer source. Mass spectra were recorded in scan mode at 70eV (35-350 amu). The source temperature was 230°C.Other experimetal conditions were as those for the CPG-FID analyses.

Identification of essential oil constituents

The compounds contained in the essential oils were identified (i) by determining their retention index (RI) according to the *n*-alkane series (C5-C18) and by comparison with literature databases [16, 17], (ii) by matching their mass spectra with those in the spectrum library (NIST08.LIB mass spectral libraries of the GC-MS data system), (iii) by comparing their mass spectra with those reported in the literature [16, 17].

Antibacterial activities of the four essential oils

Bacterial strains

Seven clinical bacteria strains isolates : *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Shigella flexneri* NR518, *Shigella sonnei* NR519, *Shigella dysenteria* CPC, *Staphylococcus aureus* NR and *Escherichia coli* ATCC25922 were tested for the antibacterial activity. The bacterial strains were provided by Bei Resources to the Antimicrobial and Biocontrol Unit, University of Yaounde 1, Cameroon. These bacteria were kept at 4°C and revived 24 hours prior to each assay on Mueller-Hinton Agar (Sigma Aldrich) at 37°C.

Preparation of stock solutions of essential oils and reference antibacterials

Stock solutions of the samples were prepared at 100 mg/mL by dissolving 100 mg of essential oil en 1 mL of 10% DMSO. Amoxicillin (Sigma Aldrich) and ciprofloxacin used as positives controls were prepared in the same conditions at 1 mg/mL by dissolving 1 mg of powder en 1 mL of acidified distilled water.

Preparation of bacterial suspension

The different bacterial suspensions were prepared and adjusted to 0.5 McFarland standard turbidity (corresponding to an approximate concentration of $1.5x10^8$ CFU/mL). To prepare these bacterial suspensions, youth cultures of bacterial colonies from 24-hour cultures on Mueller-Hinton agar (MHA) were used.

Principe of method

The inhibition effect of the essentials oils was evaluated by determining the Minimum Inhibitory Concentrations (MICs) using the microdilution method as described by CLSI (CLSI 2012, protocol M07-A9) [18]. The principal of microdilution method is based on the capacity of a microorganism to grow in a medium supplemented or not with antimicrobial substances. MICs are revealed by rezasurine colorimetry method that the principle is based on the reduction of blue resazurin to pink resorufin by the enzyme's dehydrogenases of viable cells.

Determination of Minimum Inhibitory Concentrations

The minimum inhibitory concentrations were determined using the microdilution method as described by CLSI in protocol M09 A7 en 2012 [18] coupled with a resazurin-based assay. 6 serial dilutions of each sample and positives controls (Amoxicillin and Ciprofloxacin) were performed to obtain different concentrations ranging from 31.25 to 2000 µg/mL and from 0.0071 to 2.5 µg/mL, respectively, and the final

volume in each well was 200 µL and the final concentration of DMSO was less than 1% with no effect on bacteria growth. The test was performed in Mueller Hinton Broth (Sigma Aldrich). The final concentration of the bacterial suspension was $5x10^5$ CFU/mL. The negative control was made with culture media and bacteria suspension while the sterility control was made with culture media alone. The plates (96 wells) were covered and incubated at 37°C for 24 hours. At the end of the incubation period, 20 µL of freshly prepared resazurin (0.15 mg/mL Sigma Aldrich) was introduced into each well, followed by incubation in the dark for 30 min. At the end of the incubation time, the MIC was defined as the smallest concentration of essential oil at which there was no change in coloration from blue to pink, corresponding to the lack of visible bacterial growth.

Results

Extraction yield of essential oils from plants

Extraction by hydrodistillation of *A. senegalensis*, *C. viscosa*, *C. schoenanthus* and *E. camaldulensis* gave a pale yellow essential oil in each case. The essential oil yields of the four plants are shown in Table 1. *C. schoenanthus* has shown the highest yield of essential oil (1.8%), followed by *E. camaldulensis* (1.3%), *A. senegalensis* (0.3%) and *C. viscosa* (0.02%).

Table 1: Extraction yield of essential oils

Plants	Family	Color (HEs)	Extraction yield of essential oils (%)
A. senegalensis	Annonaceae	Pale yellow	0.3
C. viscosa	Capparaceae	Pale yellow	0.02
C. schoenantus	Poaceae	Pale yellow	1.8
E. camaldulensis	Myrtaceae	Pale yellow	1.3

Analysis of essential oils from the four plants

The results of gas chromatography-mass spectrometry analysis of the essential oils from the four plants : *A. senegalensis*, *C. viscosa*, *C. schoenanthus* and *E. camaldulensis* are presented in the table 2. The compounds are listed in order of elution from the OV101 column. The essential oils of *Annona senegalensis* and *Cleome viscosa* plants are rich in sesquiterpene, while the essential oils of *Cympobogon schoenanthus* and *Eucalyptus camaldulensis* are rich in monoterpene.

Table 2: Chemical composition of the essential oils of the four plants.

Retention time	Kovat index	Compounds	Percentage (%)			
			C.S	C.V	A.S	E.C
	1002	**2-carene**	**20.48**			
2.043		*β*-cymene			2.52	
7.741		elemene			3.25	
5.041		benzene				3.41
12.686		1-methyl-4-methylene-2-(2-methyl-1-propen-1-yl)-1-vynylcycloheptane		2.08		
4.895		α-phellandrene	0.11			43.05
7.976		caryophyllene		1.53	26.07	
13.117		2-benzenedicarboxylic acid, mono (2-ethylhexyl) ester		6.35		
8.064		Aromadendrene				2.58
8.370		α-aromadendrene				3.48
8.412		γ-elemene				16.65
8.192		1, 5, 9, 9-tetramethyl-1,4,7-cycloundecatriene			22.80	
5.084		Limonene	4.94			3.41
8.333		(-)-D-germacrene			6.95	
8.938		Caryophyllene oxyde	0.20	5.67	18.75	
	1031	1,8-cineole	0.13			
	1084	fenchone	0.10			
8.950		(-)-globulol				9.46
9.005		cubenol		19.58		4.89
7.780		α-Cubebene		3.14		
	1123	Trans pinene hydrate	1.23			
	1177	Terpineol-4	0.17			
	1189	α-terpineol	1.03			
	1196	Cis-piperitol	0.39			
	1425	β-caryophyllene	1.10			
	1253	piperitone	63.61			
8.188		1, 5, 9, 9-Tetramethyl-1, 4, 7-cycloundecatriene		2.81		
	1443	α-farnesene	0.09			
	1457	Trans-β-farnesene	0.20			
8.516		δ-cadinene	0.18	26.73		
9.220		γ-Cadinene		5.19		
12.617		(-)-β-Elemene		2.76		
9.089		3, 4-dimethyl-3-cyclohexenyl methanal			14.51	
9.386		Eudesm-7(11)-en-4-ol			5.14	
	1651	β-eudesmol	0.79			
5.122		Eucalyptol				5.64
5.322		1,4-cyclohexadiene				1.90
8.217		(+)-Epibicyclosesquiphellandrene		4.71		

AS: *Annona senegalensis*; CV: *Cleome viscosa*; CS: *Cympobogon schoenanthus* and EC: *Eucalyptus camaldulensis*. The figures bellow present the peaks of chromatography analysis of the essential oils

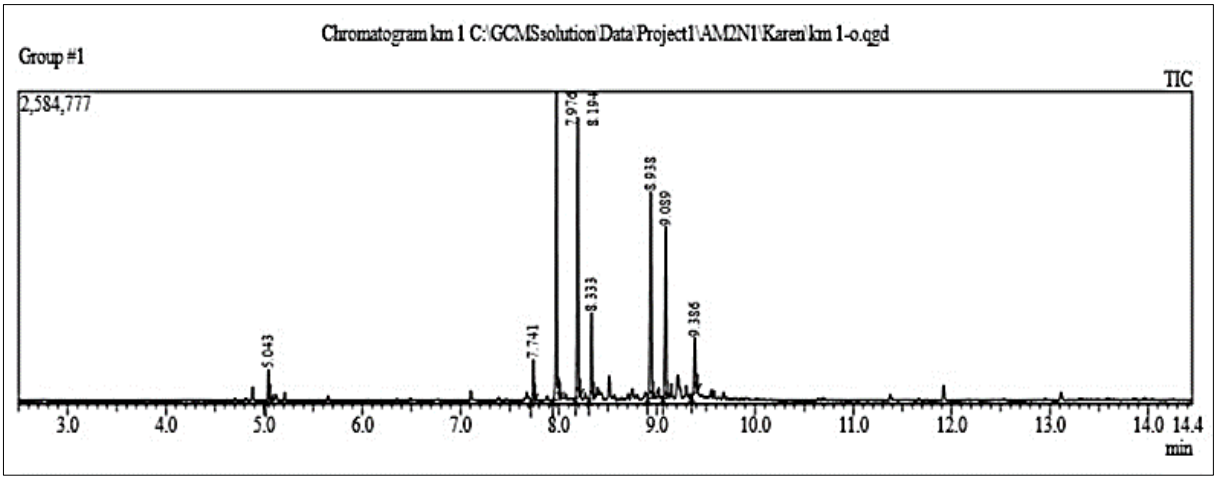

Fig 1: Retention index the constituents of the essential oil of *A. senegalensis*

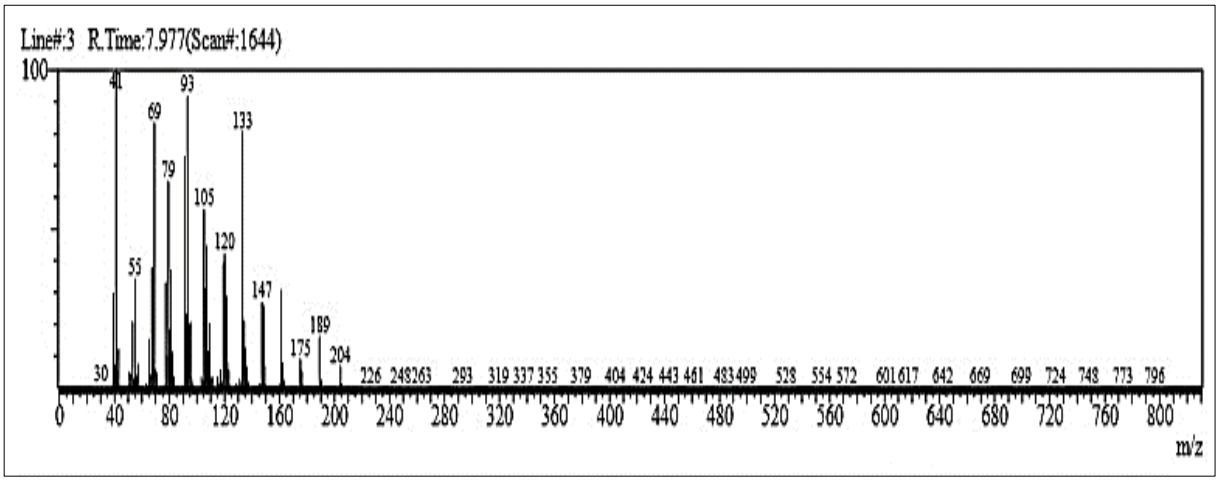

Fig 2: Mass Spectrum of the major compounds from the essential oil of *A. senegalensis*

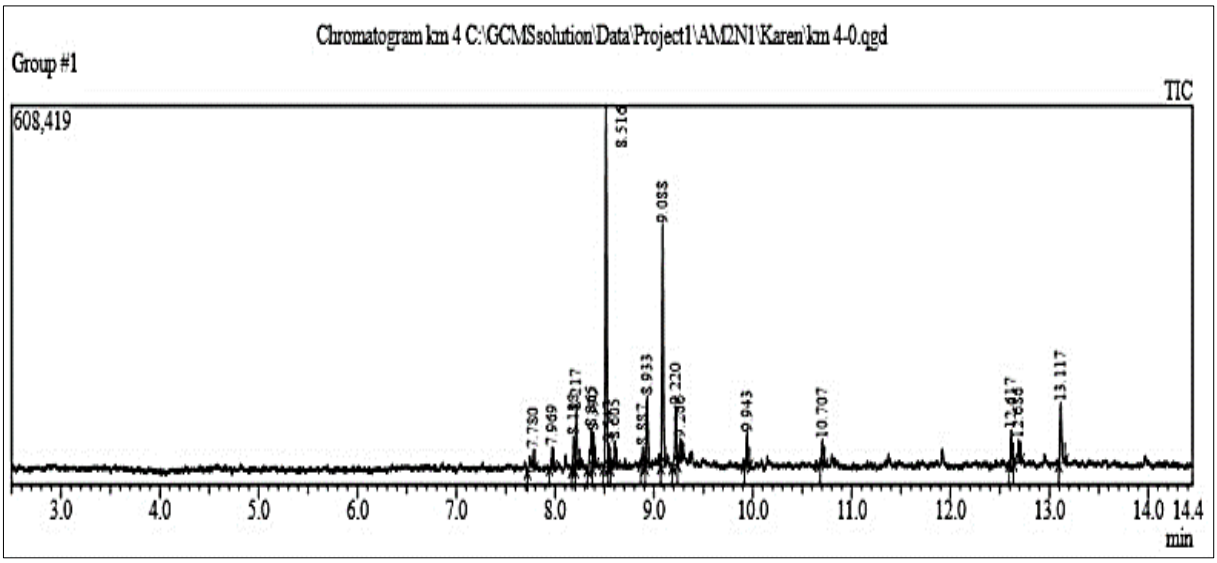

Fig 3: Retention index the constituents of the essential oil of *C. viscosa*

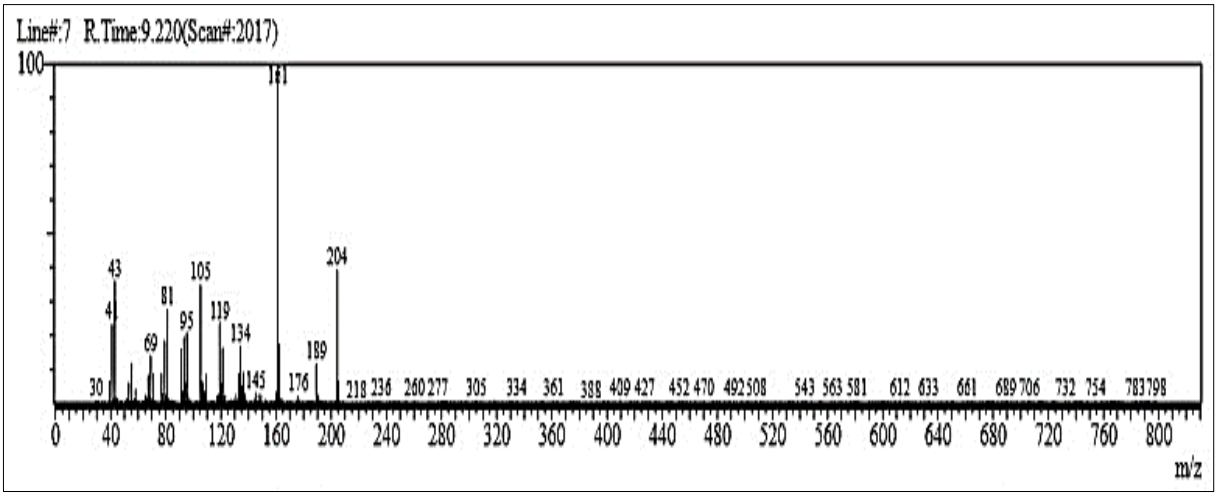

Fig 4: Mass Spectrum of the major compounds from the essential oil of *C. viscosa*

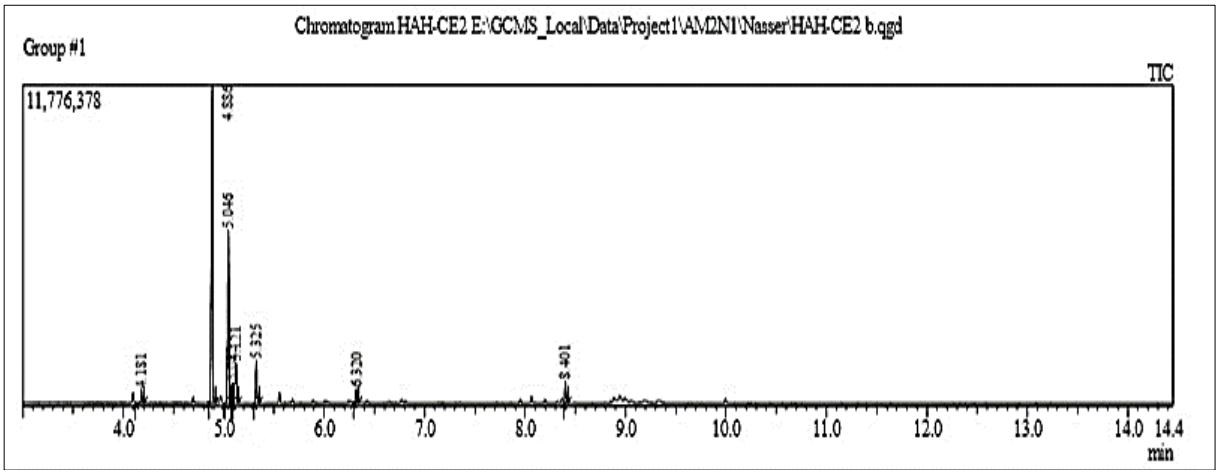

Fig 5: Retention index the constituents of the essential oil of *E. camaldulensis*

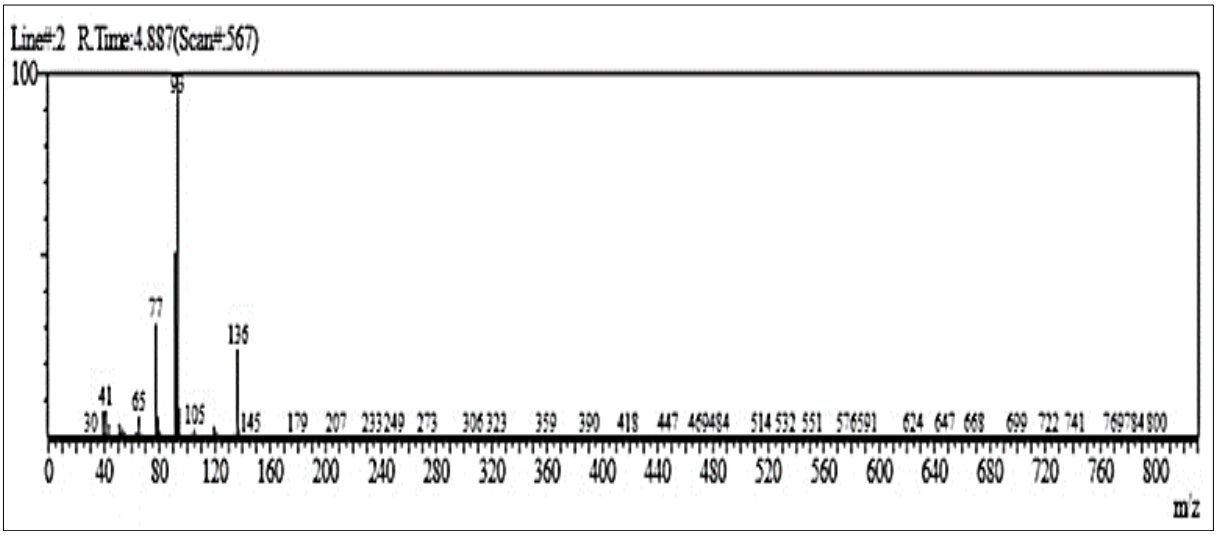

Fig 6: Mass Spectrum of the major compounds from the essential oil of *E. camaldulensis*

Antibacterial activity of essential oils from four plants

This study aimed to evaluate the antibacterial properties of essential oils of four plants from the traditional pharmacopeia of Niger. The threshold used for the antibacterial activity is that established by Tamokou *et al.* en 2017 [19], (highly active: MIC ≤100 µg/mL, significantly active: 100 < MIC ≤ 512 µg/mL, moderately active: 512 < MIC ≤ 1024 µg/mL, low activity: 1024 < MIC ≤ 2048 g/mL, and considered not active: MIC>2048 mg/mL)) showed that the activity of essential oils ranged from low activity to moderate activity. The antibacterial activity results are grouped in the table below. The MICs is varying from 500 µg/mL to 2000 µg/mL.

Table 3: Minimum Inhibitory concentrations of the essential oils of the four plants

Extracts	Minimum Inhibitory Concentrations (µg/mL)						
	Bacteria strains						
	SA NR	KP NR	AC NR	SFNR 518	SONR 519	SD CPC	EC ATCC 25922
A. senegalensis	250	NA	125	125	250	125	125
C. viscosa	2000	NA	2000	2000	2000	2000	2000
C. schoenanthus	250	250	NA	NA	500	250	250
E. camaldulensis	2000	NA	2000	2000	NA	1000	NA
Cipro	0.43	NT	0.43	0.87	0.976	1.75	0.43
Amoxicillin	0.976	NT	NT	0.976	1.952	0.976	0.976

AC NR: *Acinetobacter baumannii* NR, KP NR: *Klebsiella pneumoniae* NR, SFNR 518: *Shigella flexneri* NR518, SONR 519: *Shigella sonnei* NR519, SD CPC: *Shigella dysenteria* CPC, SA NR: *Staphylococcus aureus* NR, EC ATCC 25922: *Escherichia coli* ATCC25922, CPC: *Centre Pasteur du Cameroun*. NA: Non Active ; NT: Not Tested

Discussion

Essential oils constitue a large range of plant oils that are highly aromatic. They are found mainly in the flowers, buds, leaves, twigs, bark, wood, roots, rhizomes, bulbs, fruits, peels, seeds, and resin of plants. A few of them are obtained from animal sources or are produced by microorganisms. They are composed of a wide variety of natural organic components with different functional groups and molecular structures. Actually, different retention index systems based on different stationary phases have been used in determining essential oil constituents; by comparison of their retention indices and mass spectra [20-22]. Essential oils consist of liphatic hydrocarbons, monoterpenoids, sesquiterpenoids, and diterpenes. Other groups of compounds include phenylpropanoids, acids, alcohols, ketones, aldehydes, and fatty acids and their esters. The present study shows that the essential oil of *C. schoenanthus* is rich in piperitone and 2-carene with 63.61% and 20.48% respectively. The essential oil of *C. viscosa* is rich in *δ*-cadinene (26.73%) and cubenol (19.58%). Cubenol is also present in the essential oil of *E. camaladulensis* with a percentage of 4.89%, which is rich in α-phellandrene (43.05%) and γ-elemene (16.65%). The constituent δ-cadinene is simultaneously present in the essential oils of *C. schoenanthus* (0.18%) and *C. viscosa*. The essential oil of *A. senegalensis* is rich in caryophyllene (26.07%), 1, 5, 9, 9-tetramethyl-1,4,7-cycloundecatrien (22.80%), Caryophyllene oxyde (18.75%) and 3,4-dimethyl-3-cyclohexenyl methanal (14.51%). Caryophyllene oxyde is present in the essential oil of three species *C. schoenanthus*, *C. viscosa* and *A. senegalensis*. The main components in some Eucalyptus species (*E. bicostata*, *E. cinerea*, *E. leucoxylon*, *E. maidenii*, *E. odorata*, *E. sideroxylon*, *E. astringens* and *E. lahmannii*) were 1,8-cineole, cryptone, α-pinene, p-cymene, α-terpineol, trans-pinaocarveol, phellandral, cuminal, globulol, limonene, aromadendrene, spathulenol and terpinen-4-ol [23]. In the present study 1,8-cineole, p-cymene, trans-pinaocarveol, cryptone, cuminal, sapthulenol, α-pinene and α-terpineol were absent in the essential oil of *E. camaldulensis*. Our results shown that the major component in the essential oil of *E. camaldulensis* is α-phellandrene. Other studies on the essential oils of *E. cinerea*, *E. sideroxylon*, *E. bicostata*, *E. maidenii*, *E. leucoxylon*, *E. lehmannii* and *E. astringens*, have reported 1,8-cineole as the major component of these oils [23-25].

The essential oils of many plant species have been reported by others authors to possess useful biological, pharmacological, and therapeutic activities. The antimicrobial efficiency of the four essential oils was determined by measuring the minimum inhibitory concentration (MIC), as shown in the Table 3. Among the tested essential oils in this study, the essential oil of *A. senegalensis* was discovered to demonstrate strong antimicrobial activity. The recorded MIC on *Shigella dysenteriae*, *Shigella flexneri* and *Escherichia coli* was 125 µg/mL. According to the criteria used in this study the essential oil of *A. senegalensis* is the most active with a significant activity against 6 strains of bacteria (*A. baumannii*, *S. aureus*, *S. dysenteriae*, *S. sonnei*, *S. flexneri* and *E. coli*), but this essential oil is inactive on *Klebsiella pneumoniae.* The essential oil of *C. viscosa* is inactive on *Klebsiella pneumoniae* and have a low activity with MIC at 2000 µg/mL against 6 strains of bacteria, namely *A. baumani*, *S. aureus*, *S. dysenteriae*, *S. sonnei*, *S. flexneri* and *E. coli.* Among the four samples screened for their antibacterial activity one essential oil of *C. schoenanthus* is active against *Klebsiella pneumoniae* with MIC at 250 µg/mL, which is a significant activity. This essential oil is the less active against the other bacterial strains. According to previous study the antibacterial activity of essential oil could be due to the high mean percentage of the monoterpene hydrocarbons especially p-cymene [26]. It has been reported the high sensitive character of *S. aureus* to essential oils with a high content of p-cymene [26]. This could justify the best antibacterial activity of *A. senegalensis* which is the only sample to content p-cymene. Elaissi *et al.* in 2012 investigated the antibacterial activity of several *Eucalyptus* species and their correlation with chemical composition [23]. The main chemical compounds were determined to be 1,8-cineole, spathulenol, *a*-pinene, p-cymene, and limonene. These results are different from those in the present study with the absence of p-cymene in the essential oil of *E. camaldulensis*. Of note that among the species of the genus *Ecaluptus* studied by Elaissi *et al.* en 2012, *E. camaldulensis* was not investigated [23]. Thus, the best antibacterial activity was recorded against *S. aureus* and *E. coli*, while the correlation between the levels of active compounds in essential oil and the antibacterial activities was noticed [23, 27]. *S. aureus* is the most sensitive bacterial strain, the four samples are active against this strain with MIC ranging from 250 to 2000 µg/mL.

Conclusion

This study was designed to evaluate the antibacterial activity of essential oils of four plants from the traditionnal pharmacopeia of Niger. The essential oil of *A. senegalensis* showed the best antibacterial activity against 6 strains of bacteria, namely *A. baumannii*, *S. aureus*, *S. dysenteriae*, *S. sonnei*, *S. flexneri*, and *E. coli*. While the essential oil of *C. viscosa* is the less active. The GC-MS analysis showed that the essential oils of *A. senegalensis* and *C. viscosa* are rich in

sesquiterpene, and these of *C. schoenanthus* and *E. camaldulensis* are rich in monoterpene. All these results could justify the use of these plants in traditional medicine to cure infectious diseases, and demonstrate that these plants contain bioactive compounds. The results show also the promising possibility of using the essential oils of these plants as desinfectants. Then, further studies are needed to isolate the compounds responsible of the antibacterial activity.

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Author Contributions
Conceptualization, HAZ, DHAR, MIIA and IAT; Investigation, HAZ, MIIA and IAT; Validation, IAT and JLP; Methodology HAZ, MIIA and IAT; Bioassays, HAZ and MIIA; Writing-original draft, HAZ, MIIA and IAT; Spectral analysis, DHAR, JLP; Supervision, IAT and JLP. Manuscript editing&correction, DHAR, BNA, ASA, IAT, All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate : Not applicable

Competing interests
The authors state that there are no competing interests.

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