Original Research Article

Chemical composition and biological activity of *Guarea cedrata* (A. Chev.) Pellegr. leaf and root bark essential oil

ABSTRACT

Aims: This study aims, on the one hand, to establish the chemical composition and to evaluate the antioxidant potential of essential oils (EO) from leaf and root bark of *Guarea cedrata*, and on the other hand, to determine the antimicrobial activity of essential oils from leaf.
Methodology: EOs were analyzed by a combination of GC (Ir), GC-MS and ^{13}C NMR. The antibacterial and antifungal activity of the essential oils have been determined then the antioxidant activity has also been evaluated.
Results: 40 hydrocarbon (19.8%) and oxygenated (73.3%) sesquiterpenes were identified from root bark EO, representing 93.1% of the total composition, and 55 compounds were identified from the leaf EO, representing 91.5% of the total composition, with 58.1% hydrocarbon sesquiterpenes, 32.4% sesquiterpenes oxygenates and 1.0% hydrocarbon monoterpenes. An evaluation of the antioxidant potential of these EOs revealed a moderate antiradical activity of the EO of *G. cedrata* leaf compared to quercetin. The leaf EO tested on bacteria and yeasts showed bacteriostatic activity against bacterial strains of *Escherichia coli*, *Salmonella typhimirium*, *Bacillus subtilus* and *Candida albicans* yeasts at a concentration of 6.3 mg/ml and bactericidal activity at a concentration of 50 mg/ml.
Conclusion: This study highlighted the chemical composition, the antiradical and biological activity of the essential oils of the organs of guarea cedrata.

Keywords: Guarea cedrata, essential oil, ^{13}C NMR, GC-MS, antioxidant, antimicrobial and antifungal activities.

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1. INTRODUCTION

In recent decades, the use of medicinal plants in therapy has become increasingly popular. Due to the development of resistance to many drugs in microorganisms and their ineffectiveness, as well as the growing desire to use environmentally friendly products, more and more people are choosing natural methods of treatment and prevention [1]. Due to the presence in its composition of many bioactive compounds (mono- and sesquiterpenes, phenolic derivatives), essential oils (EO) have a wide range of pharmacological activities: antibacterial, fungicide, anti-inflammatory [2-6].
There are many aromatic plants, whose chemical composition and therapeutic activity of their EO are little or not studied. However, they may be important new sources of bioactive compounds. For example, *Guarea cedrata* is a tropical woody plant belonging to the Meliaceae family. It is one of the species of the genus Guarea, producing a wide range of secondary metabolites including limonoids, triterpenes, diterpenes and sesquiterpenes. According to the literature, these are the main components of EOs of the genus Guarea [7]. *G. cedrata*, also known as "Bossé", is a large forest tree, easily recognized by its pale grey-brown bark, winding concentric ridges and strong cedar scent. The stem reaches a height of 40 m and a diameter of up to 2 m. The crown is dense, spherical, with twisted branches [8].
G. cedrata is often used in traditional medicine to treat various conditions such as abdominal pain, food poisoning, gonorrhea, rheumatism, postpartum hemorrhage, leprosy, and as a poultice for kidney pain [9]. Studies carried out on the bark of *G.cedrata* by John A. Akinniyi and al, have made it possible to isolate three new triterpenoids: the acid 3,4-secotirucalla-4(28),7,24-trièn-3,21 -dioic acid, its methyl ester and 2'-hydroxyrohitukin [10]. The methanolic extract of the trunk bark of this species is a food attractant for adult *Sitophilus granarius* L. (insect food pest) [11]. Chantal Menut and al have shown that *G. cedrata* trunk bark EO is composed exclusively of sesquiterpenes, comprising 97.1% of the volatile composition, including 68.0% hydrocarbons and 29.1% oxygenated components. The

main constituents are p-caryophyllene (45.1%) and globulol (11.1%) [8]. To our knowledge, only one article has been published on the chemical composition of EO from the trunk bark of *G. cedrata* [8], and not a single article has been published on the chemical composition of EO from leaf and root bark. Therefore, the purpose of this work was (i) to study the chemical composition of the EO of leaf and root barks, (ii) to evaluate the antioxidant potential and (iii) to determine the antimicrobial and antifungal activities of EO from the leaf of *G. cedrata*.

2. MATERIAL AND METHODS

2.1. Plant material and EO extraction

Leaf and roots of *G. cedrata* were harvested on July 13, 2019 in Adiopodoumé (5°20'12" N and 4°7'57" W) in the district of Abidjan. The identification of this species was confirmed by the systematic botanist Dr Malan Djah François, NANGUI ABROGOUA University, Côte d'Ivoire. The plant organs were dried at room temperature for 7 days and subjected to hydrodistillation using a Clevenger-type still for 4 hours. The oil samples were dried over anhydrous $MgSO_4$ and stored in the refrigerator (4°C) in hermetically sealed brown glass bottles until analysis.

2.2. GC analysis

0.5 µL of sample solutions containing 50 µL of EO in 350 µL of $CDCl_3$ are injected and analyzed. The analyzes were carried out on a Clarus 500 PerkinElmer chromatograph (PerkinElmer, Courtaboeuf, France), equipped with a Flame Ionization Detector (FID) and two fused silica capillary columns (50 m × 0.22 mm, thickness of 0.25 µm film), BP-1 (polydimethylsiloxane) and BP-20 (polyethylene glycol). (PerkinElmer, Courtaboeuf, France) (FID) and two fused silica capillary columns (50 m × 0.22 mm, film thickness 0.25 µm), BP-1 (polydimethylsiloxane) and BP-20 (polyethylene glycol). The oven temperature was programmed from 60°C to 220°C at 2°C/min, then maintained at isothermal temperature at 220°C for 20 min; injector temperature: 250°C; detector temperature: 250°C; carrier gas: hydrogen (0.8 mL/min); distribution: 1/60; volume injected: 0.5 µL. The retention indices (RI) were determined relative to the retention times of the n-alkane series (C8–C29) by linear interpolation ("Target Compounds" software from PerkinElmer).

2.3. GC-MS analysis

0.5 µL of the sample solutions containing 50 µL of EO in 350 µL of $CDCl_3$ are injected and analyzed using a Clarus SQ8S PerkinElmer TurboMass detector (quadrupole) directly connected to a Clarus 580 PerkinElmer Autosystem XL (PerkinElmer, Courtaboeuf, France), equipped with an Rtx-1 (polydimethylsiloxane) fused silica capillary column (60 m × 0.22 mm i.d., film thickness 0.25 µm). The temperature of the oven was programmed from 60 to 230°C at a rate of 2°/min, then the isothermal temperature was maintained for 45 min; injector temperature, 250°C; ion source temperature, 250°C; carrier gas He (1 mL/min); split ratio, 1:80; injection volume, 0.2 µL; ionization energy, 70 eV. Electron ionization (EI) mass spectra were obtained in the mass range 35–350 Da.

2.4. Analysis by ^{13}C NMR

The ^{13}C NMR spectra were recorded on a Fourier transform spectrometer Bruker AVANCE (Bruker, Wissembourg, France) 400 operating at 100.623 MHz, equipped with a 5 mm probe. 40 mg of EO are dissolved in 0.5 mL of $CDCl_3$; all chemical shifts relating to the internal tetramethylsilane (TMS). The ^{13}C NMR spectra of oil samples were recorded with the following parameters: pulse width = 4 µs (rotation angle 45°); relaxation time D1 = 0.1 s, acquisition time = 2.7 s for a 128K datasheet with a spectral width of 25,000 Hz (250 ppm); CPD mode decoupling; digital resolution = 0.183 Hz/pt. The cumulative number of scans was 3000 for each sample.

2.5. Identification of compounds

The identification of the individual components was carried out: (i) by comparison of their retention indices (RI) in GC on polar and apolar columns [12]; (ii) computer correspondence with commercial mass spectra libraries [12-15]; (iii) by comparing the signals of the 13C NMR spectra of the samples

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with those of the reference spectra compiled in the laboratory's spectral library, using software developed in the laboratory [16, 17].

2.6. Evaluation of antioxidant activity

The in vitro antioxidant activity was evaluated using a spectrophotometer by measuring the scavenging capacity of the DPPH$^{\bullet}$ (1,1-diphenyl-2-picrylhydrazyl) radical according to the method described by Blois [18] with a slight modification. DPPH$^{\bullet}$ is dissolved in absolute ethanol to give a solution at a concentration of 0.003 mg/mL. Different ranges of concentrations of EOs are prepared in absolute ethanol (5 mg/mL; 2.5 mg/mL; 1.25 mg/mL; 0.625 mg/mL; 0.313 mg/mL; 0.156 mg/mL). In dry and sterile tubes, are introduced 0.5 mL of prepared extracts and 0.75 mL of ethanolic solution of DPPH$^{\bullet}$. After an incubation period of 30 min at laboratory temperature and protected from light, the absorbance is read at 517 nm compared to a control sample prepared from 1.25 mL of ethanolic solution of DPPH$^{\bullet}$. The reference positive control is quercetin prepared under the same conditions as the extracts to be tested. The percentage reduction (PR) of the DPPH$^{\bullet}$ radical by the extracts was calculated according to the following formula:

$$\mathbf{PR\ (\%) = [(A_{DPPH} - A_e) / A_{DPPH}] \times 100}$$

A_{DPPH}: absorbance of DPPH•; **A_e:** absorbance of the extract

The effectiveness of the samples against DPPH$^{\bullet}$ was assessed by graphical determination of the CR_{50} (concentration that reduces 50% of DPPH$^{\bullet}$) [19].

2.7. Determination of antibacterial and antifungal activity

2.7.1. Microbial and fungal strains and growth conditions

The following microbial and fungal strains were used for this test: GRAM (-) bacteria: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimirium* SO 66; GRAM (+): *Staphylococcus epidermidis* CIP. 53124, *Staphylococcus aureus* CIP 4.83, *Bacillus subtilus* ATCC 6633 and yeasts: *Candida albicans* ATCC 10231, *Candida tropicalis* ATCC 13803, *Candida glabrata* ATCC 66032. They were subcultured into tubes with preservative agar (in the central puncture) and incubated at 37°C. After 24 hours of incubation, these tubes are stored at -80°C and at refrigeration temperature (2±1°C). In order to always have strains available, transplants of refrigerated strains are carried out every 15 days. Before carrying out the antibacterial and antifungal tests for each strain, two consecutive subcultures are carried out. They were first inoculated in Mueller Hinton broth and incubated for 24 h at 37 ± 1°C.
The second subculture is carried out on solid medium (nutrient agar) one day before the antibacterial and antifungal test. The whole was incubated at 37± 1°C for 18 hours so that the bacterial and fungal cells were in the exponential growth phase. Several colonies are picked from this fresh bacterial and fungal culture and mixed with sterile distilled water. To standardize the loading of the initial inoculum, we used the method of comparing the bacterial (fungal) density to the density of a Mc Farland reference tube (0.5) whose loading was assumed to be 105 CFU/mL.

2.7.2. Well microdilution method

The antibacterial activity and the antifungal activity of different EOs at different concentrations (50 mg/mL; 25 mg/mL; 12.5 mg/mL; 6.25 mg/mL; 3.13 mg/mL; 1, 56 mg/mL; 0.78 mg/mL; 0.39 mg/mL) were determined for each strain by the method of Berghe and Vlietinck [20], from a culture of 18 to 20 h (105- 106 CFU/mL). The seeding of the 1 mL inoculum is carried out on the surface of the Mueller-Hinton medium previously poured into Petri dishes. After 15 min, the wells were excised with Pasteur pipettes (thick end 6 mm). The bottom of the wells is sealed with a drop of Mueller-Hinton agar to limit the diffusion of oils under the agar. Then, 50 µl of oil at different dilutions and a reference (gentamicin or amphotericyn B respectively for fungi and bacteria) are distributed in each well. After diffusion, the cultures are incubated in ovens at 37° C. for 24 h. Inhibition rings are measured with a vernier caliper. The activity is considered nil for an inhibition diameter (i.d.) less than or equal to 8 mm; weak for d.i. of 8 and 14 mm, average for i.d. 14 and 20 mm; strong for d.i. greater than or equal to 20 mm.

2.7.3. Determination of the Minimum Inhibitory Concentration (MIC)

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the technique used consists in seeding the EO with a standardized inoculum in a range of decreasing concentrations (50 mg/mL; 25 mg/mL; 12.5 mg/mL; 6.25 mg/mL; 3.13 mg/mL; 1.56mg/mL; 0.78mg/mL; 0.39mg/mL). After incubation, monitoring the range gives access to the Minimum Inhibitory Concentration (MIC), which corresponds to the lowest HE concentration capable of inhibiting bacterial (fungal) growth after 18 to 24 hours of contact.

3. RESULTS AND DISCUSSION

3.1. Chemical composition

G. cedrata yielded EO of yellowish colored Leaf and roots barks of *G. cedrata* were harvested on July 13 and orange colored root barks in yields of 0.22% and 0.39% (m/m based on fresh plant material) respectively. The combination of GC (RI), GC-MS and ^{13}C NMR analysis methods applied to the two EO samples identified 55 compounds in leaf EO and 40 compounds in root barks EO, representing 91.5% and 93.1% of the total composition, respectively. These two EOs are dominated by sesquiterpene compounds. The major compounds of Leaf and roots of *G. cedrata* were harvested on July 13 EO are: β-selinene (22.9%), α-selinene (15.2%) and β-elemene (8.1%). Two other compounds are present in significant proportions: β-eudesmol (4.6%) and α-eudesmol (4.6%). Monoterpenes are present in proportions of less than 1%: the most abundant is limonene (0.6%). This composition differs quantitatively from those of the EOs of leaf of several species of Guarea from Brazil. Indeed, *G. scabra* leaf EO is dominated by *cis*-caryophyllene (33.37%) and *trans*-α-bergamotene (11.88%) while the species *G. silvatica* is mainly composed of caryophyllene epoxide (36.54%) and counts among its compounds a diterpene (kaurene) [7] and *G. Guidonia* leaf EO is dominated by eudesm-6-en-4β-ol (21.0%), guai-6-en-10β-ol (21.0%) and eudesma-5,7-diene (19.2%) [21, 22].
Root bark EO is dominated by viridiflorol (55.5%), carotol (5%) and 11-α-himachal-4-en-1-β-ol (4.0%) are present in appreciable proportions. The most abundant hydrocarbon sesquiterpene in root bark EO is *allo*-aromadendrene (2.7%). No monoterpene has been identified in this EO. Despite the fact that these two EOs are rich in sesquiterpenes, they have different chemical compositions. Leaf contain a greater amount of hydrocarbon sesquiterpenes (58.14%), characterized by β-selinene, while the root barks are richer in oxygenated sesquiterpenes (73.3%) with viridiflorol as representative. No compound present in appreciable proportion is common to the two samples. The chemical compositions of the EOs from leaf and root barks of *G. cedrata* are different from those of the EO from the trunk barks of this p-caryophyllene dominated species [8].
It is important to note that in general, EOs of Guarea species do not contain monoterpenes. Thus, the presence of a monoterpene in the leaf EO is unusual. However, its low proportion compared to sesquiterpenes confirms previous results according to which the predominance of hydrocarbon and oxygenated sesquiterpenes and the almost total absence of monoterpenes is a marker of EOs of the Guarea genus [1].

Table 1: chemical composition of EO from leaf and root bark

Compounds	RIa	RIp	Relative proportion (%)	
			leaf	root bark
α-pinene	931		0,1	-
β-pinene	971	1 116	0,3	-
limonene	1 022	1 205	0,6	-
α-longipinene	1 351	1 469	-	0,7
(+)-himachala-2,4-diene	1 358	1 597	-	0,9
cyclosativene	1 369	1 483	-	0,8
longicyclene	1 372	2 166	-	0,2
α-copaene	1 375	1 500	0,4	0,2
daucene	1 379	1 493	-	0,4
β-élémene	1 387	1586	8,1	0,4
***iso*-caryophyllene**	1 403	1 576	0,4	0,8

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α-ionone	1 407	1 849	0,1	-
α-bergamotene cis	1 410	1 569	-	0,1
(E)-β-caryophyllene	1 416	1 591	2	0,4
β-gurjunene	1 420	1 603	-	0,1
dauca-5,8-diene	1 431	1 612	-	1,6
***trans*-α-bergamotene**	1 432	1 580	0,3	0,1
α-guaiene	1 434		0,3	-
α-humulene	1 449	1 669	2,4	0,4
***allo*-aromadendrene**	1 457	1 645	-	2,7
carota-1,4-diene	1 464	1 650	-	1,0
drima-7,9(11)-diene	1 465	1 680	0,6	-
selina-4,11-diene	1 470	1 676	3,1	-
β-curcumene	1 475	1 757	-	0,9
β-selinene	1 482	1 715	22,9	0,1
ledene	1 490	1 695	-	1,0
α-selinene	1 491	1 725	15,2	-
α-germacrene	1 492		-	2,5
β-himachalene	1 495	1 708	-	1,1
α-bulnesene	1 498	1 718	0,4	0,3
β-bisabolene	1 502	1 721	-	0,1
γ-cadinene	1 505	1 744	0,3	-
γ-humulene	1 508	1 731	-	2,4
***trans*-calamenene**	1 508	1 832	0,1	-
***7-epi*-α-selinene**	1 511	1 762	0,9	-
γ-cadinene	1 513	1 757	0,2	-
***ar*-himachalene**	1 522	1 696	0,1	0,7
selina-4(15),7(11)-diene	1 528	1 804	0,2	-
elemol	1 533	2 077	2,8	-
selina-3,7(11)-diene	1 536	1 777	0,3	-
***(E)*-nerolidol**	1 547	2 040	0,2	-
spathulenol	1 563	2 119	0,1	1,8
caryophyllene oxide	1 569	1 978	0,6	2,0
globulol	1 574	2 070	0,2	0,8
guaiol	1 584	2 086	3	-
viridiflorol	1 584	2 083	-	55,5
carotol	1 587	2 014		5
ledol	1 592	2 024	-	0,3
humulene oxide II	1 593	2 010	0,8	-
β-himachalene oxide	1 596	1 986	-	0,3
neo intermedeol	1 598	2 133	1	-
1,10-di-epi cubenol	1 614	2 060	-	0,1
eremoligenol	1 614	2 182	0,9	-
cubenol	1 617	2 034	-	0,8
γ-eudesmol	1 617	2 164	2,7	-
caryophylla-4(12),8(13)-dien-5- α-ol	1 619	2 292	0,5	-

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daucol	1 620	2 287	-	0,5
hinesol	1 624	2 206	0,4	-
τ-cadinol	1 625	2 173	-	0,2
α-muurolol	1 628	1 727	-	0,9
τ-muurolol	1 628	2 182	0,6	-
cubenol	1 630	2 034	0,3	-
11α-himachal-4-en-1β-ol	1 633	2 103	-	4,0
β-eudesmol	1 634	2 224	4,6	-
pogostol	1 637	2 194	-	1,0
intermedeol	1 637	2 247	2,9	-
α-eudesmol	1 639	2 215	4,6	-
eudesm-11-en-4-α-ol	1 642	2 301	0,9	-
bulnesol	1 651	2 198	2,0	-
ledene oxide II	1 700	2 279	1,0	-
α-cyperone	1 724	2 338	2,1	-
valerenal	1 742	2 210	0,2	-
neophytadiene	1 835	1 925	0,1	-
hydrogenated monoterpenes			1,0	-
hydrogenated sesquiterpenes			58,1	19,8
oxygenated sesquiterpenes			32,4	73,3
total			91,5	93,1

The order of elution and the percentages are given on apolar column. RIa and RIp: retention indices measured respectively on apolar (BP-1) and polar (BP-20) column.

3.2. Antioxidant activity

Figure 1 shows the results of the antioxidant test, *in vitro*, of EOs from the leaf and root bark of *G. cedrata*, against the DPPH$^{\bullet}$ radical, using quercetin as a reference. From these results, it can be seen that the potential for DPPH$^{\bullet}$ radical reduction by EOs gradually increases with increasing EO concentration, similarly for quercetin. The same is true for quercetin. However, percentage reductions of DPPH$^{\bullet}$ by EO are lower than those of quercetin for all the concentrations used.

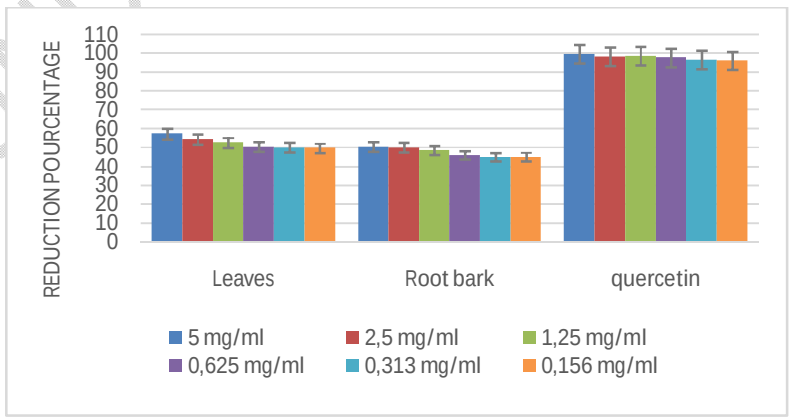

Figure 1: Antioxidant profiles of *G. cedrata* leaf and root bark EOs and quercetin

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In order to better assess the power of reduction of $DPPH^{\bullet}$ by the samples tested, the RC_{50}, ie the concentration which reduces the radical activity of $DPPH^{\bullet}$ by 50%, was determined graphically by linear regression (Table 2). Indeed, the lower the value of the RC_{50}, the higher the activity [23].
G. cedrata leaf EO shows antioxidant activity with a RC_{50} of 0.227 mg/mL about 10 times higher than that of the EO of root bark. The antioxidant activity of the EO of *G. cedrata* leaf can be considered as very moderate with regard to the value of the RC_{50} of quercetin (0.005mg/mL), used as a reference.

Table 2: RC_{50} of *G. cedrata* leaf and root bark EOs samples

	Quercetin	Leaf EO	Root bark EO
RC_{50} (mg/mL)	0,005	0,227	2,137

3.3. Antibacterial and antifungal activities

The results of the measurements of inhibition diameters of antibacterial and antifungal tests are recorded in table 3.
The antimicrobial potential of *G. cedrata* leaf EO was determined by measuring the diameters of the zones of inhibition and determining the minimum inhibitory concentrations (MIC). For the antibacterial tests, the greatest diameter of inhibition was obtained with very sensitive *Escherichia coli* (i.d = 16 mm), followed by *Bacillus subtulis* and *Salmonella typhimirium* (i.d = 9mm for each strain). Concerning the antifungal tests, an inhibition diameter of 9 mm was obtained for *Candida albicans*. All other microbial strains: yeasts; *Candida tropicalis*, *Candida glabrata* and bacteria; *Pseudomonas aeruginosa*, *Staphylococcus epidermidis* and *Staphylococcus aureus* demonstrated resistance to the EO of *G. cedrata* leaf.
The MIC determined against the sensitive strains all have the same value (6.25 mg/mL). This MIC value is significantly higher than that of the reference antibiotic and antifungal.

Table 3: inhibition diameters of leaf **EO on bacteria and fungi (mm)**

	strains	inhibition diameters (mm)		
		Leaf EO	Gentamicin	Amphotericin B
Antifungal tests	***Candida albicans***	13		30
	Candida tropicalis	0		30
	Candida glabrata	0		30
Antibacterial tests	***Bacillus subtulis***	09	35	
	Staphylococcus aureus	0	35	
	Staphylococcus epidermidis	0	35	
	Pseudomonas aeruginosa	0	30	
	Salmonella typhimirium	09	25	
	Escherichia coli	16	33	

GRAM (-): Escherichia coli ATCC 25922; Pseudomonas aeruginosa ATCC 27853; Salmonella typhimirium SO 66. GRAM (+) : Staphylococcus épidermidis CIP. 53124 ;Sstaphylococcus aureus CIP 4.83 ; Bacillus subtilus

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ATCC 6633. YEASTS/ MOLDS: Candida albicans ATCC 10231; Candida tropicalis ATCC 13803 ; Candida glabrata ATCC 66032.

The values of MIC of *G. cedrata* leaf EO determined against the yeast *Candida albicans* and the bacteria *Bacillus subtulis*, *Salmonella typhimirium* and *Escherichia coli* are indicated in **table 4**.
MIC values are generally consistent with inhibition diameters. The fungicidal or bactericidal activity could be due to the presence of sesquiterpenes in the EO of *G. cedrata* leaf, in particular eudesmol, an oxygenated compound with strong antimicrobial activity [24].

Table 4: Antibacterial and antifungal parameters of *G. cedrata* leaf EO

Strains	Leaf EO	Amphotericin B	Gentamicine
	MIC (mg/mL)	MIC (mg/mL)	MIC (mg/mL)
Candida albicans	**6,25**	**0,0003**	
Bacillus subtilus	**6,25**		**0,0007**
Salmonella typhimirium	**6,25**		**0,003**
Escherichia coli	**6,25**		**0,003**

4. CONCLUSION

The analysis of EOs from leaf and root bark of *G. cedrata* by GC (RI), GC-MS and ^{13}C NMR showed that these EOs are very rich in sesquiterpenes. Root bark EO is dominated by viridiflorol while leaf EO is represented by the β-selinene/α-selinene couple. Leaf EO showed better antioxidant activity than root bark EO against DPPH$^{\bullet}$, but this activity was moderate compared to quercetin. Leaf EO has also demonstrated antifungal activity against *Candida tropicalis* and antibacterial activity against *Bacillus subtulis*, *Salmonella typhimirium*, and *Escherichia coli*. Nevertheless, these antimicrobial capacities are average. The antioxidant and antimicrobial potentials of *G. cedrata* leaf EO are certainly moderate but constitute significant assets in the use of this species.

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