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

E-ISSN: 2278-4136
P-ISSN: 2349-8234
Impact Factor (RJIF): 6.35
www.phytojournal.com
JPP 2025; 14(6): 227-233
Received: 02-10-2025
Accepted: 05-11-2025

Bala Namata Abba
(1) Laboratory of Natural Substances and Organic Synthesis, Faculty of Sciences and Techniques, Abdou Moumouni University, Niamey B.P 10662, Niger
(2) Department of Fundamental Sciences, National School of Engineering and Energy Sciences, Agadez University, Agadez B.P 199, Niger

Amadou Tidjani Ilagouma
Laboratory of Natural Substances and Organic Synthesis, Faculty of Sciences and Techniques, Abdou Moumouni University, Niamey B.P 10662, Niger

Abderrahmane Romane
Laboratory of Applied Chemistry and Biomasse, Faculty of Sciences Semlalia, Cadi Ayyad University, 40000 Marrakech B.P 2390, Morocco

Corresponding Author:
Bala Namata Abba
(1) Laboratory of Natural Substances and Organic Synthesis, Faculty of Sciences and Techniques, Abdou Moumouni University, Niamey B.P 10662, Niger
(2) Department of Fundamental Sciences, National School of Engineering and Energy Sciences, Agadez University, Agadez B.P 199, Niger

Chemical composition and antimicrobial potential of essential oil and ethanolic extract of *Ocimum canum* Sims. against eleven pathogenic bacteria

Bala Namata Abb, Amadou Tidjani Ilagouma and Abderrahmane Romane

DOI: https://www.doi.org/10.22271/phyto.2025.v14.i6c.15662

Abstract
The objectives of the present study were to investigate the chemical composition and to evaluate the antioxidant and the antibacterial activities of the essential oil and ethanolic extract of *Ocimum canum* Sims. grown in Niger. Based on the Gas chromatography-mass spectrometry analysis, 47 compounds were identified representing 99.66% of the total oil. The antioxidant activity of the essential oil and ethanolic extract was assayed by using the free radical-scavenging activity (2,2 diphenyl-1-picrylhydrazyl: $DPPH^{\cdot}$) and the ferric reducing antioxidant power assay. The ethanolic extract has demonstrated a good antioxidant potential while the essential oil showed a moderate antioxidant potential with both methods. Agar disc diffusion and broth microdilution assays were used to evaluate the antibacterial activity of the essential oil and ethanolic extract of *Ocimum canum* against Eleven pathogenic bacteria. The essential oil exhibited a better antibacterial activity than the ethanolic extract, especially against multi-resistant *Acinetobacter baumannii* P1483 with MIC and MBC values of 0.03 mg/mL and *Enterococcus faecium* H3434 with MIC value of 0.03 mg/mL and MBC value of 0.06 mg/mL.

Keywords: *Ocimum canum*, Essential oil, Ethanolic extract, Chemical composition, Antioxidant activity, Antibacterial activity

Introduction
known as *Ocimum americanum* Linn. [1], *Ocimum canum* Sims. belongs the Lamiaceae family, containing approximately, 200 species of herbs and shrubs. This plant species is widely distributed in tropical and southern Africa, China, and India; naturalized in southern Europe and Australia, and tropical South America [1]. *Ocimum canum* is a pubescent erect much-branched herb having 15 to 60 cm high with sub-quadrangular striate branches. Leaves are elliptic-lanceolate, entire or faintly toothed, glabrous, and gland-dotted. Flowers are white, pink, or purplish in elongate racemes with more or less closely set whorls [2]. According to the literature, various parts of *Ocimum canum* are traditionally used for treating various diseases, such as fever, cold, skin diseases, dysentery, diabetes, indigestion, toothache, headaches, coughs, diarrhea, constipation, microbial infections, and kidney malfunction [3, 4]. It is also a rich source of aroma compounds and essential oil containing variable bioactive constituents. The essential oil obtained from this plant were shown to possess potential antimicrobial and antioxidant activities [5, 6]. Although, several studies have reported various biological activities of *Ocimum canum* essential oil [7, 8]. However, to the best of our knowledge, there are no data reported on the chemical composition, antioxidant and antibacterial activities of *O. canum* grown in Niger. Therefore, as part of our ongoing research on the valorization of aromatic and medicinal plants from Niger, this study aimed to investigate the chemical composition and evaluate the antioxidant activity as well as the antibacterial activity of the essential oil (*EO*) and ethanolic extract of *O. canum*.

Materials and Methods
Plant material and essential oil extraction
The *Ocimum canum* samples were collected from Boureimi, located in the South of Niger (Altitude 251 m, Latitude (N) 13°06'003", Longitude (E) 003°31'476") during October 2024. A botanist, Professor Mahamane Saadou, Department of Biology at the Faculty of Sciences and Technology, Abdou Moumouni University of Niamey, Niger, identified the plant and a voucher number (NA/07) was given to the plant. The plant materials were air-dried at room

temperature away from light. The essential oil (*EO*) was obtained by hydrodistillation using a Clevenger type apparatus [9]. A hundred (100) g of dried aerial parts of the plant were hydrodistilled for three hours. The obtained essential oil was stored under refrigeration (4°C) until analysis.

Ethanolic extract preparation

The ethanolic extract was prepared as follow: 40 grams of powder from the aerial parts of the plant were placed in 200 mL of ethanol and left under agitation for 24 hours at room temperature. After 24 hours, the mixture was filtered on filter paper, and the solvent was evaporated to dryness with a rotary evaporator (BUCHI Rotavapor R-210) at 50 °C. The dry residues obtained were weighed and stored until analysis.

GC-MS/FID analysis of essential oil

The essential oil (*EO*) analysis was carried out by GC-MS/FID technique using a GCMS-QP2010SE Gas Chromatograph Mass Spectrometer (SHIMADZU CORPORATION, Columbia-USA), equipped with two columns, a Zebron ZB-5ms (20 m x 0.18 mm x 0.18 µm) and Zebron ZB-WAX (20 m x 0.18 mm x 0.18 µm), and coupled with a Mass Analyzer single quadrupole system, operating in Scan/SIM mode. Injector temperature was at 280 °C, and the sample was injected by using split mode (1/30), the volume injected was 1 µL. Helium was used as carrier gas (1 mL/min). The initial temperature of the columns was maintained at 50 °C for 3 min and increased at 2 °C/min until 280 °C, and then maintained at 280 °C for 30 min. The system operated in the electron impact (EI) mode, and data were collected at a mass range of 20 to 500, scan time 0.2 seconds. GC-FID analysis was performed under the same experimental conditions as described for the GC-MS. Constituents were identified by comparing their retention indices (RI) determined by using a homologous series of n-alkanes and mass spectral fragmentation patterns with those stored in the NIST08.LIB mass spectral libraries of the GC-MS data system and from the literature data [10]. The concentrations of the chemical components were calculated based on GC peak areas without using correction factors.

Antioxidant activity

The antioxidant activity of the essential oil and ethanolic extract was evaluated using DPPH• radical-scavenging activity method and ferric reducing power antioxidant assay (FRAP). Ascorbic acid was used as a positive control in both assays.

DPPH• radical-scavenging activity

The essential oil and ethanolic extract antioxidant property was investigated using DPPH• radical-scavenging assay according to the method described by Blois [11] with slight modifications. The EO and ethanolic extract were diluted to prepare a range of concentrations from 0.1 to 1 mg/mL. 1 mL of each concentration of the samples was mixed with 2 mL of DPPH• (0.2 mmol) ethanolic solution. Ascorbic acid was prepared in the same way as the positive control. Each assay was performed in triplicate. The prepared samples were incubated in the dark at room temperature for 30 min. After incubation, the absorbance of the mixture was measured at a wavelength of 517 nm using a spectrophotometer Uviline 9400 (SECOMAM) against a blank solution (1 mL of ethanol + 2 mL of DPPH• solution). The antioxidant activity of all the test samples was expressed as IC_{50} (mg/mL), defined as the concentration of the antioxidant needed to scavenge 50% of DPPH• present in the test solution. The percentage of inhibition of DPPH• free radical-scavenging activity was calculated by the followed equation:

DPPH• scavenging effect (%) = $\mathbf{[(A_0-A_1)/A_0] \times 100}$

Where A_0 is the absorbance of the blank solution and A_1 is the absorbance of the test sample.

Ferric reducing antioxidant power assay (FRAP)

The essential oil and ethanolic extract ability to reduce Fe^{3+} was determined according to the method reported by Yıldırım *et al*. [12]. The concentrations range from 0.1 to 1 mg/mL of the samples and ascorbic acid were prepared with absolute ethanol. Then 1 mL of each test samples was added to 2.5 mL of phosphate buffer (0.2 M, pH = 6.6) and 2.5 mL of $[K_3Fe(CN)_6]$ solution (1%). The mixture was incubated at 50 °C in a water bath (DIGITERM 100, J.P. SELECTA, S.A; Spain) for 30 min. After incubation, the solution was left at room temperature and 2.5 mL of trichloroacetic acid (10%) was added, and then centrifuged (HERMLE Z323K, Germany) at 3000 rpm for 10 min. Finally, 2.5 mL of the upper layer solution was mixed with 2.5 mL of distilled water and 0.5 mL of $FeCl_3$ (0.1%). The absorbance was measured at 700 nm (A_{700}) using a spectrophotometer Uviline 9400 (SECOMAM). The antioxidant activity of all the tested samples were expressed as EC_{50} (mg/mL), which corresponds to the effective concentration of the samples giving an absorbance of 0.5 for reducing power, and was determined by linear regression analysis of the graphs of A_{700} against the corresponding samples concentrations [13].

Antibacterial activity

The essential oil and ethanolic extract were screened for antibacterial activity against eleven pathogenic bacterial strains including Gram-negative: multi-resistant *Acinetobacter baumannii* P1483, Extended-spectrum β-lactamase (ESBL)-*Escherichia coli* Bu8566, *Salmonella* spp. H1548, *Proteus mirabilis* Bu190, *Enterobacter cloacae* Bu147, *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli (*ATCC 25922), *Klebsiella pneumoniae* (ATCC 700603) and Gram-positive: methicillin-resistant *Staphylococcus aureus* P1123, *Enterococcus faecium* H3434 and *Staphylococcus aureus* (ATCC 25923). Evaluation of the antibacterial activity was carried out using the agar disc diffusion assay, and the microdilution method as reported in our previous study [14].

Results and Discussion

Chemical composition of the essential oil

The yield of the essential oil (EO) obtained from the aerial parts of *O. canum* by hydrodistillation was 0.9%. The results of the GC/MS analysis of the EO are reported in Table 1. The chemical composition analysis leads to the identification of 47 compounds from *O. canum* EO. The identified components represented 99.66% of the total oil constituents. The most abundant compound of the EO was 1,8-cineole with 22.94% of the total peak area. The other major components were camphor (21.81%), bornyl acetate (10.57%), α-zingiberene (5.18%), α-terpineol (4.36%), (*E*)-β-caryophyllene (3.95%), and *trans*-α-bergamotene (3.95%). The EO was dominated by oxygenated monoterpenes, which comprised about 56.23% of the total composition, followed by sesquiterpene hydrocarbons representing 16.78%. Monoterpene hydrocarbons (10.41%) and oxygenated sesquiterpenes (3.25%) were the minor chemical classes of compounds

identified in the EO. Previous studies have reported the chemical composition of *O. canum* EO from other countries. Our results are in accordance with those reported by Djibo *et al*. [15], who have found 1,8-cineole (45.1-59.9%), camphor (8.1-59.9%), β-pinene (4.2-5.3%), α-terpineol (3.5-4.1%), *trans*-α-bergamotene (2.2-2%), and β-caryophyllene (1.9-1.7%) as major compounds of *O. canum* leaves EO. Also, Wangrawa *et al*. [16], reported 1,8-cineole (44.6%), camphor (15.9%), α-pinene (7.1%), and β-pinene (5.1 %) as main compounds of *O. canum* leaves EO, harvested from Burkina Faso. Besides, other studies have reported EO of *O. canum* predominated by 1,8-cineole from Brazil [17] and from India [18]. According to the review of the published literature, *O. canum* EO presents qualitative and quantitative variability in its chemical compositions, characterized by a wide range of major compounds [8, 19, 20]. We noticed that citral, eugenol, methyl chavicol, and thymol which have previously been found in substantial amounts in the EO of *O. canum*, were not detected in the EO of *O. canum* aerial parts from Niger. This variability observed in the chemical composition of *O. canum* EO from different origins could be due to the environmental and climatic conditions, geographical origin, the used part of the plant, seasonal variation, the vegetative stage, genetic background, morphogenic factors, the harvest time as well as by other factors that can influence the chemical composition of the essential oil [21, 22]. Then, the present study happened to be the first report on the chemical composition of *O. canum* EO from Niger.

Table 1: Chemical composition of *Ocimum canum* essential oil

No.	RI[a]	RI[b]	[c]Compounds	Percentage (%)
1	948	939	α-Pinene	2.76
2	956	954	Camphene	1.26
3	973	979	β-Pinene	1.58
4	985	990	Myrcene	0.73
5	998	1002	δ-2-Carene	0.26
6	1025	1024	*p*-Cymene	0.66
7	1038	1029	Limonene	2.20
8	1039	1029	β-Phellandrene	0.48
9	1039	1031	1,8-Cineole	22.94
10	1048	1045	Ocimene quintoxide	0.12
11	1058	1059	γ-Terpinene	0.35
12	1082	1088	Terpinolene	0.13
13	1092	1096	Linalool	1.93
14	1108	1098	*trans*-Sabinene hydrate	0.15
15	1109	1112	1-Octen-3-yl acetate	0.68
16	1114	1122	Myrcenol	0.82
17	1141	1146	Camphor	21.81
18	1158	1149	Camphene hydrate	0.22
19	1168	1177	Terpinen-4-ol	1.90
20	1187	1183	*p*-Cymen-8-ol	0.12
21	1191	1188	α-Terpineol	4.36
22	1238	1232	*exo*-Fenchyl acetate	0.26
23	1259	1258	2-Phenyl ethyl acetate	0.53
24	1277	1285	Bornyl acetate	10.57
25	1346	1342	*p*-Menth-8-ene-1,2-diol	0.65
26	1343	1346	Myrtenyl acetate	2.04
27	1374	1376	α-Copaene	0.29
28	1417	1419	(*E*)-β-Caryophyllene	3.95
29	1430	1434	*trans*-α-Bergamotene	3.95
30	1440	1442	(*Z*)-β-Farnesene	0.40
31	1459	1454	α-Humulene	0.32
32	1458	1456	α-Patchoulene	0.19
33	1491	1493	α-Zingiberene	5.18
34	1494	1500	α-Muurolene	0.21
35	1508	1505	(*E*,*E*)-α-Farnesene	0.51
36	1526	1522	β-Sesquiphellandrene	0.66
37	1519	1523	δ-Cadinene	0.88
38	1534	1532	(*Z*)-Nerolidol	0.57
39	1537	1546	Selina-3,7(11)-diene	0.24
40	1576	1578	Spathulenol	0.44
41	1580	1583	Caryophyllene oxide	1.08
42	1580	1590	Globulol	0.18
43	1590	1592	Viridiflorol	0.20
44	1625	1634	(3*Z*)-Hexenyl phenyl acetate	0.12
45	1645	1642	epi-α-Muurolol	0.12
46	1637	1646	Cubenol	0.40
47	1757	1761	**(***Z***)**-Lanceol	0.26
Sub-totals (%)				
Monoterpene hydrocarbons 10.41				
Oxygenated monoterpenes 56.23				

Sesquiterpene hydrocarbons	16.78
Oxygenated sesquiterpenes	3.25
Other components	12.99
Total identified (%)	99.66

RI[a]: experimental retention indices.
RI[b]: retention indices from literature.
[c]Compounds are listed in order of elution from the column.

Antioxidant activity

The results of antioxidative capacity of *O. canum* essential oil and ethanolic extract assessed by using DPPH• radical-scavenging activity method and their ability to reduce Fe^{3+} to Fe^{2+} are presented in Table 2.

Table 2: DPPH• free radical-scavenging activity (IC_{50}) and Ferric reducing antioxidant power (EC_{50}) of essential oil and ethanolic extract of *Ocimum canum*

Tested samples	Assay	
	DPPH•: IC_{50} (mg/mL)	**Ferric reducing power: EC_{50} (mg/mL)**
Essential oil	4.15	4.01
Ethanolic extract	0.61	0.70
Ascorbic acid	0.044	0.028

The ethanolic extract exhibited a good free radical scavenging activity (DPPH•), with an IC_{50} value of 0.61 mg/mL, while the essential oil was less active, reflected by a high IC_{50} value (4.15 mg/mL). But both samples are significantly less active than the reference compound, ascorbic acid (IC_{50} = 0.044 mg/mL). In the same way, a good reducing power activity (FRAP) of the ethanolic extract was recorded having an EC_{50} value of 0.70 mg/mL. The essential oil expressed a low reducing power activity (EC_{50} = 4.01 mg/mL). The ethanolic extract and essential oil of *Ocimum canum* exhibited a lower Ferric reducing antioxidant power comparing to the standard ascorbic acid (EC_{50} = 0.028 mg/mL). The antioxidant activity of the ethanolic extract and essential oil was moderately significant in both test systems studied.

In the literature, other authors have reported the antioxidant activity of *Ocimum canum* species from different countries. Selvi *et al.* [23] found in their study a better DPPH- radical scavenging activity of the essential oil of *Ocimum canum* harvested in India than ours with an IC_{50} of 523.55 ± 0.001 µg/mL. However, our results are higher than that reported by Bunrathep *et al.* [24], on the EO of *O. canum* aerial parts from Thailand, they found an EC_{50} of 8485.29 mcg/mL by using DPPH• method. Another study conducted by Dinata *et al.* [25] on the ethanolic extract of leaves of the Indonesian species showed significant DPPH- radical scavenging activity with an IC_{50} of 80.55 ppm. We have observed a variability of the antioxidant activity according to the origin of the plant. This variation in the antioxidant activity of the ethanolic extract and essential oil of the *Ocimum canum* may be due to the nature, concentration and structures of their chemical constituents [26].

Antibacterial activity

The antibacterial activity of the essential oil and ethanolic extract of *Ocimum canum* was evaluated against eleven pathogenic bacteria by using the agar disc diffusion assay and the microdilution method.

Agar disc diffusion assay

The bacteria sensibility to the essential oil, the ethanolic extract, and Chloramphenicol as a positive control was estimated by the diameter of the inhibition zones as described by Ponce *et al.* [27]. The results are presented in Figure 1. The EO and ethanolic extract of *O. canum* exhibited variable levels of antibacterial efficiency against all the microorganisms tested. On the eleven strains tested, multi-resistant *Acinetobacter baumannii* P1483 and *Enterococcus faecium* H3434 were found to be the most sensitive strains to the EO, with inhibition zones diameters of 36 ± 2 mm and 34 ± 1 mm respectively. However, *Salmonella* spp. H1548, *Klebsiella pneumoniae* ATCC 700603, and *Pseudomonas aeruginosa* ATCC 27853 were not sensitive to the EO of *O. canum.* The results showed that all the Gram-positive bacteria were sensitive to the EO, while some Gram-negative bacteria have not shown any sensitivity to the EO. These findings indicated that the EO of *O. canum* is more active against the Gram-positive bacteria than the Gram-negative ones, and are in accordance with the literature data indicating that the Gram-positive bacteria seemed to be more sensitive to essential oils than Gram-negative bacteria [28]. The ethanolic extract of *O. canum* was not active against *Proteus mirabilis* Bu190, ESBL-*E Coli* Bu8566, *Enterobacter cloacae* Bu147 and *MRSA* P1123. The higher inhibitory diameter for the ethanolic extract was obtained for *MRAB* P1483 (16 ± 1 mm). Chloramphenicol was more active than the EO and ethanonnlic extract of *O. canum* against Gram-negative bacteria. The highest inhibition zone for Chloramphenicol was obtained for *Salmonella* spp. H1548 (30 ± 1 mm). The antibacterial potency observed for the EO of *O. canum* in the present study may be related to its major constituents such as (*E*)-β-caryophyllene, 1,8-cineole, camphor, bornyl acetate, and α-terpineol, which have been reported to have antibacterial properties [29, 30]. Moreover, minor components, could also contribute by synergistic effects to the obtained antibacterial activity [31]. The antibacterial potential of our EO is comparable to what has been previously reported on the EO of *O. canum* species originated from other regions [28]. Nascimento *et al.* [32], related low antibacterial activity of *O. canum* aerial parts EO against *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922), they reported diameter of inhibition zone with a value of 9 mm for both microorganisms by using the method of diffusion in agar. The variation of the antibacterial activity of *O. canum* EO observed according to the results of the present work and those reported by other authors, could be correlated to the chemical composition variability of this species [30, 31].

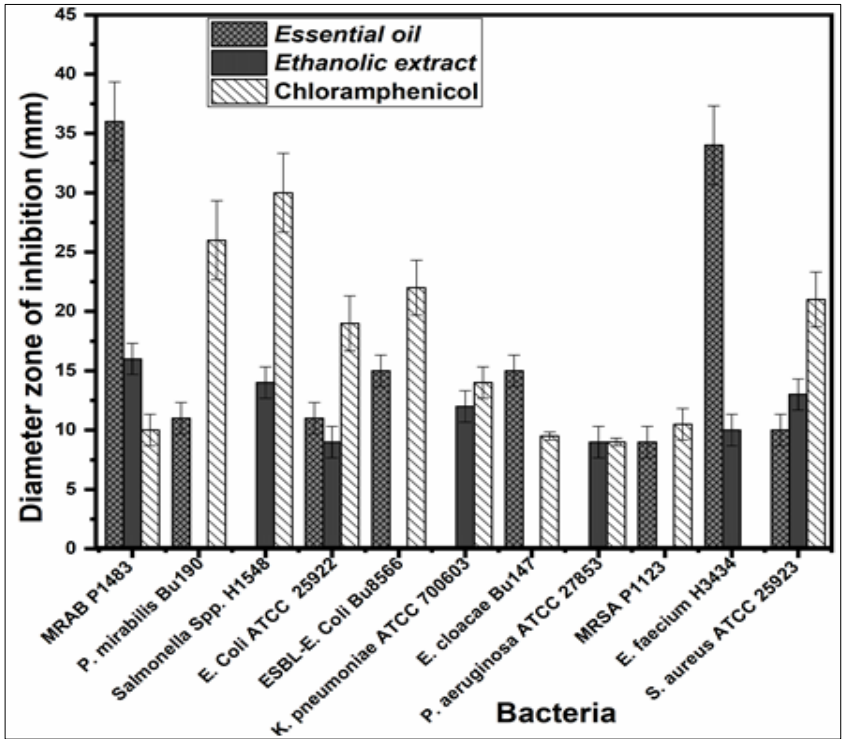

Fig 1: Zone diameter of inhibition (mm) from the action of *Ocimum canum* essential oil, ethanolic extract and Chloramphenicol against the pathogenic bacteria tested (Mean ± S.D.).

MRAB, multi-resistant *Acinetobacter baumannii; E. cloacae*, *Enterobacter cloacae*; *E. coli*, *Escherichia coli*; *E. faecium*, *Enterococcus faecium*; ESBL, extended-spectrum β-lactamase; *K. pneumoniae*, *Klebsiella pneumoniae;* MRSA, methicillin-resistant *Staphylococcus aureus*; *P. aeruginosa*, *Pseudomonas aeruginosa*; *P. mirabilis*, *Proteus mirabilis*; *S. aureus*, *Staphylococcus aureus*.

Microdilution assay

The results of antibacterial activity of the essential oil and ethanolic extract of *Ocimum canum*, and Chloramphenicol as a positive control by using the microdilution assay are presented in Table 3. The analysis of those results show that the essential oil has a varied spectrum of inhibitory and bactericidal activity, with MIC and MBC values ranging from 0.03 to 10 mg/mL. The *MRAB* P1483 strain was the most sensitive to the essential oil, with MIC and MBC values of 0.03 mg/mL, followed by *Enterococcus faecium* H3434, whose sensitivity is expressed by an MIC value of 0.03 mg/mL and an MBC value of 0.06 mg/mL. The ethanolic extract showed varying levels of effectiveness against the bacteria, with MIC and MBC values ranging from 1.25 to 10 mg/mL. However, these MIC values for the essential oil and ethanolic extract of *Ocimum canum* are lower than those for the positive control, Chloramphenicol. Other studies in the literature have reported similar results to ours. Yayi-Ladekan *et al.* [33], have also reported the antibacterial activity of *O. canum* leaves EO harvested between 7 AM and 7 PM, against *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25923, *Acinetobacter baumannii* ATCC 19609 and resistant *Staphylococcus aureus* 1199B NorA, and obtained a range of MIC's values from 0.38 to 7.19 mg/mL. The antibacterial activity of *O. canum* leaves EO have been reported by Da Silva *et al.* [20], they found MIC's values of 200, 250, and 100 µg/mL for *Escherichia coli* 25922, *Staphylococcus aureus* 12600, and *Pseudomonas aeruginosa* 27853, respectively. Oyedemi *et al.* [3] evaluated the antibacterial activity of the methanolic extract of *O. canum* leaves from Nigeria on the strains *Staphylococcus aureus* NCTC 12981, *Staphylococcus aureus* NCTC-13373, *SARM* 274829, E-*SARM*-15, S. aureus ATCC-25923 and *SA*-1199B. Their results demonstrated the sensitivity of these bacteria with MICs ranging from 256 to 512 mg/L. Thus, we note a difference in the sensitivity of some bacteria to the essential oil or extract depending on the origin of the plant.

Table 3: MIC and MBC values from the action of *Ocimum canum* essential oil, ethanolic extract and Chloramphenicol against the pathogenic bacteria tested.

Bactéries	Essential oil		Ethanolic extract		Chloramphenicol
	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)
MRAB P1483	0.03	0.03	1.25	2.5	2.5
Proteus mirabilis Bu190	5	5	-	-	0.06
Salmonella spp. H1548	-	-	2.5	2.5	0.03
E. Coli ATCC 25922	5	5	10	>10	0.25
ESBL-*E. Coli* Bu8566	2.5	5	-	-	0.5
KP ATCC 700603	-	-	2.5	5	1.25
PA ATCC 27853	-	-	10	>10	-
Enterobacter cloacae Bu147	1.25	5	-	-	-
MRSA P1123	10	>10	-	-	2.5
Enterococcus faecium H3434	0.03	0.06	10	>10	-
Staphylococcus aureus ATCC 25923	5	10	2.5	2.5	0.5

ATCC, American Type Culture Collection; MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration; *E. Coli*, *Escherichia coli;* ESBL, Extended-spectrum β-lactamase.

Conclusion

It can be concluded that for the first time the chemical composition, antioxidant and antibacterial activities of the essential oil and ethanolic extract of *O. canum* grown in Niger were investigated. The chemical composition analysis showed that the EO is rich in bioactive compounds with 47 constituents identified representing 99.66% of the total oil, and the chemotype of the EO is 1,8-cineole type. The essential oil and the ethanolic extract have exhibited good antioxidant potential, and demonstrated a good antibacterial effect against the tested microorganisms, particularly the Gram-positive bacteria. These results showed that the EO and the ethanolic extract of *O. canum* could be considered as a natural source of bioactive compounds with potential antioxidant and antibacterial activities. These findings are very promising in having an alternative source of natural antioxidant agents, and new effective and affordable approaches to treat some infectious diseases caused by those human pathogenic bacteria. This study contributes to the valorization of aromatic and medicinal plants of Niger and provides scientific evidence for the use of *O. canum* in traditional medicine. In this paper, we report for the first time the chemical composition, antioxidant and antibacterial activities of the essential oil and ethanolic extract of *Ocimum canum* grown in Niger.

Acknowledgments

The author(s) are grateful to Professor Jean Luc-Pirat, ENSCM, Montpellier-France, for his contribution to the analysis of the essential oil, to Nabila Soraa and Ilham Dilagui, Laboratory of Microbiology and Virology, Faculty of Medicine and Pharmacy, Cadi Ayyad University, Marrakech-Morocco, for their contribution on the antibacterial tests.

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