Chemical Analysis of the Essential Oils of Three *Cistus* Species Growing in North-West of Algeria

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Summary

The study reports for the first time the chemical composition and the antibacterial activity of the essential oil hydrodistilled from three Cistaceae growing in Algeria: *Cistus ladaniferus* L., *C. albidus* L. and *C. monspeliensis* L. The oils were analyzed by GC-FID and GC-MS analyses. The major components of *C. ladaniferus* were 5-*epi*-7-*epi*-α-eudesmol (13.6%) and borneol (12.5%) whereas for *C. albidus* the main constituents were *epi*-α-bisabolol (11.4%) and β-bourbonene (8.7%). *Epi*-13-manoyl oxide (28.6%), kaur-16-ene (8.1%) and nonanal (5.4%) were the principal ones for *C. monspeliensis*. *In vitro*, antimicrobial activity of the oils was investigated against nine microorganisms by disk diffusion and agar dilution assays. The Gram-positive bacteria resulted sensitive to the three oils, especially *Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 25923. The volatiles of *C. monspeliensis* showed the best activity compared with other oils, comparable to or better than Gentamicin, a conventional antibiotic used as positive control in this study. The minimum inhibitory concentration (MIC) value of the oil was 0.25µg/L.

Key words

Cistaceae, Essential oils, GC, GC-MS, Antimicrobial activity

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Introduction

Antibiotics have been used against various infectious diseases for very long. Unfortunately, recent fast spread of new infections and the increasing resistance of microorganisms to synthetic antibiotics have driven researchers to explore various strategies to avoid therapeutic impasse. Therefore, scientific research into alternative sources of antimicrobials is necessary. Natural remedies and especially medicinal plants have been used to treat various diseases since ancient times. Plants synthesize a wide variety of bioactive compounds, among which essential oils, an important class of secondary metabolites.

Essential oils from different medicinal plants tested against Gram-positives and Gram-negatives bacteria showed an important antimicrobial activity (Benayad et al., 2013), so it is of interest to investigate medicinal plants to develop new antibacterial molecules able to overcome the resistance of microorganisms to drugs. Unfortunately, only 5-10% out of about 250.000 species are chemically exploited (Rossi et al., 2007). The Mediterranean area, due to the climate and soil conditions, is the pool of more than 10.000 medicinal and aromatic plant species (Araniti et al., 2012; Gonçalves et al., 2013).

The Cistaceae is an important family, consisting of 8 genera, with about 175 species distributed in the Northern hemisphere (temperate regions) and in South America. Most of this large biodiversity is distributed in the Mediterranean region (Iriondo et al., 1995; Attaguile et al., 2000; Başlar et al., 2002; Mahmoudi et al., 2016). They are perennial shrubs, subshrubs or herbs growing on poor soils and considered as pioneers in degraded areas (Robles and Garzino, 1998; Palá-Paúl et al., 2005; Zidane et al., 2013; Szeremeta et al., 2016). The genus *Cistus*, with its 25 species, is very represented in this geographical area being distributed from the Canary Islands to the Caucasus mountains, with particular abundance in the western Mediterranean area (Barrajón-Catalán et al., 2016; Ferrer-Gallego et al., 2013). It occurs, following climate and soil conditions, in the Iberian Peninsula, Canary Islands, Northwestern Africa, Greece and Turkey (Benjemia et al., 2013). The most abundant species in the basin are: *Cistus monspeliensis*, *C. ladaniferus*, *C. salviifolius*, *C. laurifolius* and *C. albidus* (Paolini et al., 2008). Some previous investigations on the chemical composition of the essential oils from the most representative *Cistus* species and their biological activities are present in the literature (Benayad et al., 2013; Rossi et al., 2007; Palá-Paúl et al., 2005; Zidane et al., 2013; Zidane et al., 2013; Oller-Lopez et al., 2005; Greche et al., 2009; Martos et al., 2011; Gülz, 1984; Gomes et al., 2005; Mariotti et al., 1997; Rincon et al., 2000; Verdeguer et al., 2012; Macionni et al., 2007; Angelopoulou et al., 2001; Angelopoulou et al., 2002; Loizzo et al., 2013; Hutschenreuther et al., 2010; Mohammedi and Atik, 2011).

In Algeria, the genus *Cistus* L. is represented by 10 species (Quézel and Santa, 1963): *C. ladaniferus*, *C. sericeus* (endemic to Algeria and Morocco), *C. libanotis*, *C. villosus* L., *C. heterophyllus, C. salvifolius*, *C. crispus*, *C. albidus*, *C. varius* Pourret, *C. monspeliensis.* These perennial shrubs can grow up to 1.5 m, with evergreen long leaves, purple or white flowers (with or without a red dark spot). They grow on calcareous or siliceous soils in forests, woodlands, brushwood along the coasts, in the Tell Atlas and the highlands of Algeria (Quézel and Santa, 1963). The flowering period is between April and June. Eight plant species of this genus have been recorded in Tlemcen (North-West of Algeria) according to the inventory of the National Park of Tlemcen, among them *C. ladaniferus*, *C. albidus* and *C. monspeliensis*. They are known by the local population as '*Touzzala*'. An ethnobotanical study in the region of Tlemcen reported that the leaves of *C. ladaniferus* heal wounds and can be used as decoction for the prevention and treatment of renal disorders. However, the decoctions of various species like *Thymus*, *Myrtus* associated with *C. monspeliensis* are employed to treat various skin infections. The leaves of *C. monspeliensis* are also used as antidiarrheal, anti-hemorrhagic, anti-inflammatory aid and to treat headaches as well. (Guide Illustre de la Flore Algérienne, 2012). No uses in folk medicine are known for *C. albidus* in this region. In the traditional folk medicine of Morocco, a decoction of the leaves of *C. ladaniferus* is used for its many healing properties: antidiarrheal, antispasmodic and antiacid (Zidane et al., 2013). In Greece, the infusion of the leaves of *C. monspeliensis* substitutes tea, whereas flowers are used to treat asthma (Angelopoulou et al., 2001). In the past, in Italy, the leaves of *C. albidus* were dried and used instead of tobacco (Macionni et al., 2007). Pharmacological studies on *Cistus* extracts and essential oils helped to discover new activities, such as antioxidant, antimicrobial, cholinesterase inhibitory, antiproliferative and cytotoxic ones (Benayad et al., 2013; Gonçalves et al., 2013; Zidane et al., 2013; Barrajón-Catalán et al., 2016; Benjemia et al., 2013; Angelopoulou et al., 2001; Loizzo et al., 2013; Amensour et al., 2010; Bouamama et al., 2006). The essential oils of some spices of this genus are used by the industry of perfumes and cosmetics (Robles and Garzino, 1998; Oller-Lopez et al., 2005; Greche et al., 2009). The labdanum is a resin extracted from some *Cistus* species, mainly from *C. ladanifer*. This gum is of a great economic interest, in particular as a natural fixative in perfumery, especially in Cyprus perfumes (Iriondo et al., 1995; Gomes et al., 2005).

The aim of this study is to evaluate the chemical composition and the antimicrobial activities of the essential oils from three species of this genus growing in the North-West of Algeria: *C. ladaniferus*, *C. albidus* and *C. monspeliensis*. No previous reports on the composition of the oils for the three species growing in Algeria are present in the literature, apart from one study of the antibacterial activity of the oils from *C. ladaniferus* by Mohammedi and Atik (2011). This work will contribute to get a better knowledge of the flora of Algeria.

Materials and Methods

Plant Material

C. ladaniferus, *C. albidus* and *C. monspeliensis* (Cistaceae) were collected at the flowering stage, in May 2014, in the Hafir forest [Latitude 34°46'46" N, Longitude 01°26'15" W, Altitude 1270 m] and in Ez Ziatine region [Latitude 35°7'60" N, Longitude 1°43'60" W, Altitude 319 m] near Tlemcen in the North-West of Algeria (Fig. 1). These regions are characterized by a sub-humid climate. Botanical identifications of plants were conducted by Professor Mohamed Bouazza. For each plant collected from its natural habitat, a voucher specimen was deposited in the Herbarium of the Laboratory of Ecology and Natural Ecosystem Management, University of Tlemcen.

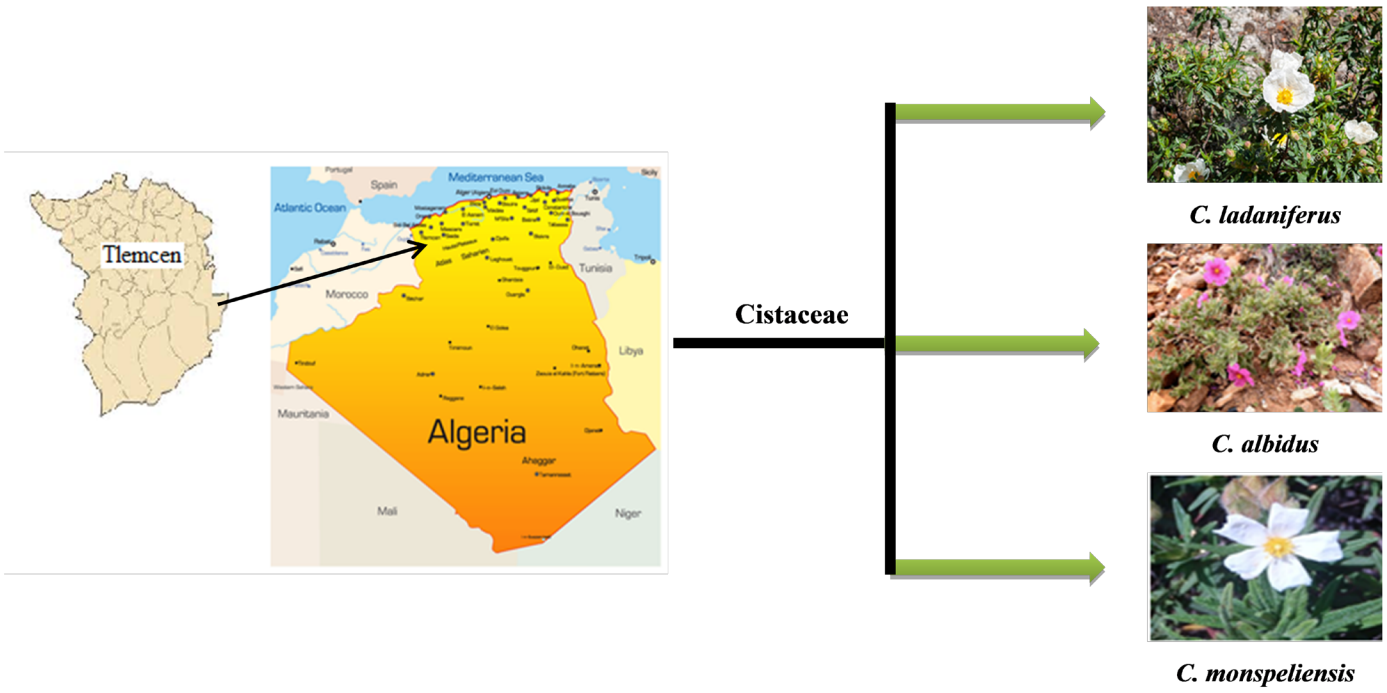

Figure 1. Location of collection sites of the three *Cistaceae* species

Isolation of Essential Oils of the Selected Plants

Essential oils were isolated from fresh aerial parts (leaves, flowers, stems) of *C. ladaniferus*, *C. albidus* and *C. monspeliensis* separately through hydrodistillation by Clevanger's apparatus for 5 h according to the method recommended by the *European Pharmacopeia* (2004). The isolated oils (yield 0.08% (w/w), 0.02% (w/w) and 0.003% (w/w) respectively) were dried over anhydrous sodium sulphate and the essential oils were stored at +4°C, in clean glass vials, until GC-MS analyses process.

Chemical Analysis

The analysis of essential oils was performed as previously reported (Chaib et al., 2017).

GC/EI-MS analyses were performed with a Varian CP-3800 gas-chromatograph equipped with a DB-5 capillary column (30 m x 0.25 mm; coating thickness 0.25 µm) and a Varian Saturn 2000 ion trap mass detector. Analytical conditions: injector and transfer line temperatures 220 and 240 °C respectively; oven temperature programmed from 60 °C to 240 °C at 3 °C/min; carrier gas helium at 1 mL/min; injection of 0.2 µL (10% hexane solution); split ratio 1:30.

GC-FID analysis was performed using a HP 5890-series II gas equipped with a HP-5 (30 m 0.25 mm ID, 0.35 mm film thickness) fused silica capillary column, carrier gas helium (1.0 mL/min); split ratio 1:30; injection 0.5 µL of 10% hexane solution. The oven temperature was programmed from 60 to 240 °C at 3 °C/min. The temperature of injector and detector were 220 and 250 °C, respectively. The identification of the components was performed by comparison of their retention times with those of pure authentic samples and by means of their linear retention indices (RI) relative to the series of *n*-hydrocarbons.

The identification of the constituents was based on comparison of the retention times with those of authentic samples, comparing their linear retention indices relative to the series of *n*-hydrocarbons, and on computer matching against commercial (NIST 2014 and Adams) and home-made library mass spectra built up from pure substances and components of known mixtures and MS literature data (Swigar and Silverstein, 1981; Davies, 1990; Adams, 1995; Joulain and König, 1998).

Antimicrobial Assays

Microorganisms and Culture Conditions

The bacterial test strains, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923, *Listeria monocytogenes* ATCC 15313, *Enterococcus faecalis* ATCC 49452, *Klebsiella pneumoniae* ATCC 70603, *Escherichia coli* ATCC 25912, *Citrobacter freundii* ATCC 8090, were maintained on Nutrient Broth (Sigma) at 37 °C for 18 h. The bacteria suspensions (10^8 CFU/mL) were spread onto Müller-Hinton Agar (MHA) (Sigma) plates. *Candida albicans* ATCC 10231, *Candida albicans* ATCC 26790 were maintained on Sabouraud dextrose broth (SDB) for 48 h, then suspensions (10^8 CFU/mL) were spread onto Sabouraud dextrose agar (Sigma) plates. Strains references were provided by the Pasteur Institute of Oran (Algeria).

Disk Diffusion Method

To evaluate the antimicrobial activities of essential oils, the agar diffusion method was employed. Sterile filter-paper disks (6 mm in diameter) were impregnated with 5µL, 15µL, 20µL, 25µL and 30µL of the essential oils (Awadh et al., 2001; Elgorashi and Van Staden, 2004), then the disks were transferred into plates and incubated during 18 to 24 h at 37 °C for bacteria and for 20 to 24 h

at 30 °C for the yeasts. The standard drugs gentamicin (10 µg/disc) and amphotericin B (200 µg/disc) were used as positive control in the tests (Ponce et al., 2003). Inhibition zones diameters (DI) were measured in millimeters and compared with those of the controls.

DI < 8 mm (resistant strains); 9 mm ≤ DI ≤ 14 mm (sensitive strains); 15 mm ≤ DI ≤ 19 mm (very sensitive strains); DI > 20 mm (extremely sensitive strains) (European Committee for Antimicrobial Susceptibility Testing, 2003).

Determination of Minimum Inhibitory Concentration (MIC)

The MIC of different essential oils was determined using the method of the microplate (96 wells) (CLSI, 2006). Starting from a stock solution of each oil dissolved in DMSO (dimethylsulfoxide), series of doubling dilution of each oil were prepared in a microplate over the range 40.00-0.08 mg/mL. Then, 198 µL of bacterial or yeast suspension (10^5 CFU/mL) were distributed into twelve wells. A volume of 2 µL of the essential oils solution was added to the eleven wells, using the last well as a control, and the final volume in each well was 200 µL. The plates were covered and incubated for 24 h at 37 °C. The MIC was defined as the low concentration of oil that inhibits visible growth.

Statistical Analysis

All tests were performed in triplicate and were reported as mean±SD using PAST3.03 program.

Results

Chemical Characterization of Essential Oils

The essential oils of the fresh aerial parts of *C. ladaniferus*, *C. albidus* and *C. monspeliensis* were obtained in low yields 0.08%, 0.02% and 0.003% (w/w), respectively. All the oils were yellow and had an unpleasant odour. The higher yield observed for *C. ladaniferus* (0.08%) is in agreement with other studies on the same species growing within the Mediterranean area. The differences can be attributed to the climatic factors that influence the production of the secondary metabolites in plants (Robles and Garzino, 1998).

In the essential oil of *C. ladaniferus*, 32 compounds were identified, representing 99.6% of the whole oil. This essential oil was composed mainly by terpenoid derivatives. The oxygenated ones were found in higher percentages (8 monoterpenes 27.5% and 9 sesquiterpenes 30.0%). Eight monoterpene hydrocarbons accounted for 25.6%, whereas six sesquiterpene hydrocarbons formed a smaller amount of the oil (12.6%). The major constituents of the oil were 5,7-di-*epi*-α-eudesmol (13.6%), borneol (12.5%), camphene (12.2%), δ-cadinene (7.6%), viridiflorol (6.4%), 4-terpineol (5.7%) and α-pinene (4.2%).

The chromatographic profile of the essential oil of the aerial parts of *C. albidus* from Algeria, showed that it is composed only of sesquiterpenes (Table 1). 31 compounds were characterized: 15 sesquiterpene hydrocarbons (48.6%) and 16 oxygenated sesquiterpenes (44.8%). The principal components were *epi*-α-bisabolol (11.4%), β-bourbonene (8.7%), *ar*-curcumene (8.3%), α-zingiberene (7.4%), γ-muurolene (5.6%), 14-hydroxy-α-muurolene (5.2%), β-caryophyllene (4.5%), 1,10-di-*epi*-cubenol (4.4%) and T-cadinol (4.0%).

The investigation carried out on the essential oils isolated from the aerial parts of *C. monspeliensis* growing in Algeria led to the identification of 37 components, representing 90.3% of the total oil. Different chemical classes have been characterized, with the total absence of the monoterpene hydrocarbons. In detail, four oxygenated monoterpenes (3.7%), two oxygenated sesquiterpenes (2.4%), three sesquiterpene hydrocarbons (3.7%), two diterpene hydrocarbons (11.4%), two oxygenated diterpenes (28.6%), ten apocarotenes (16.7%) and fourteen non-terpene derivatives (23.9%) were present. The main components of the oil are *epi*-13-manoyl oxide (28.6%), kaur-16-ene (8.1%), nonanal (5.4%), *cis*-α-ambrinol (3.3%) and *(E)*-α-ionone (3.0%).

Antimicrobial Activity

The results of the preliminary tests of the antimicrobial activity by disk diffusion showed that all the essential oils present an activity towards *Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 25923. For the other strains, the activity diverges. Indeed, by increasing the load of disks, the effect of the oils becomes evident and strains pass from the resistant to the sensitive state (Gram-negatives bacteria) and from the sensitive to the very sensitive state, and even to the extremely sensitive one (case of Gram-positives bacteria) (Table 2).

Discussions

Previous studies about the composition of the essential oil of *C. ladaniferus* and its subspecies have been reported by other authors in different countries (Rossi et al., 2007; Mariotti et al., 1997; Rincon et al., 2000). A comparative study of the essential oil chemical profile obtained for Algerian plants showed both qualitative and quantitative differences. Indeed, monoterpene hydrocarbons are represented mainly by high percentages of α-pinene in most papers, with values varying from 19.46 to 47.1% (Rossi et al., 2007; Gülz, 1984; Mariotti et al., 1997; Rincon et al., 2000), also on the basis of the plant part. In some studies in the leaves of Moroccan plants, viridiflorol is another important compound, ranging from 16.4 to 19.4% (Greche et al., 2009; Martos et al., 2011); in Portugal and Spain it reached 15.1% (Gomes et al., 2005; Verdeguer et al., 2012), while French oils were characterized by 13.59% of this oxygenated sesquiterpene (Mariotti et al., 1997). The variety *C. ladaniferus* var. *maculatus* Dun. contained 7.2% of viridiflorol in the essential oil of the leaves (Oller-Lopez et al., 2005), while smaller amounts (2.8%) were reported for plants growing in Morocco (Zidane et al., 2013). Borneol was present in small percentage in all the reports (Rossi et al., 2007; Greche et al., 2009; Martos et al., 2011; Gomes et al., 2005; Mariotti et al., 1997; Rincon et al., 2000), with the only exception of an essential oil obtained from the leaves of Moroccan plants, where it reached 11.1%, a value comparable with the present study (12.5%) (Zidane et al., 2013). Camphene was also present in comparable amounts in Moroccan samples, 12.2% (Zidane et al., 2013; Greche et al., 2009) and 15.5% (Gülz, 1984).

Some studies on *C. albidus* are present in literature and deal with the chemical composition of the essential oils obtained from different plant parts (flowers, leaves, aerial parts) and using different modes of extraction or sampling, i.e. hydrodistillation, Solid-Phase Micro Extraction, steam distillation.

Table 1. Composition (%) of *Cistus* Essential Oils from North-western Algeria

No.[a]	Compounds	RI[b]	Essential Oils (%)[c]			Identification[f]
			C. ladaniferus	*C. albidus*	*C. monspeliensis*	
1	*(E)*-3-Hexen-1-ol	853	nd[d]	nd	1.2	RI, MS
2	Tricyclene	928	**2.9**	nd	nd	RI, MS
3	*α*-Pinene	941	**4.2**	nd	nd	RI, MS
4	Camphene	955	**12.2**	nd	nd	RI, MS
5	Sabinene	977	0.6	nd	nd	RI, MS
6	6-Methyl-5-hepten-2-one	987	nd	nd	0.9	RI, MS
7	2-Methyl-2-hepten-4-one	997	nd	nd	**2.4**	RI, MS
8	*α*-Terpinene	1020	0.8	nd	nd	RI, MS
9	p-Cymene	1028	**2.2**	nd	nd	RI, MS
10	Cistus cyclohexanone (syn. 2,2,6-Trimethyl cyclohexanone)	1035	**3.9**	nd	nd	RI, MS
11	*γ*-Terpinene	1063	1.9	nd	nd	RI, MS
12	1-Octanol	1071	nd	nd	1.0	RI, MS
13	Terpinolene	1090	0.8	nd	nd	RI, MS
14	Linalool	1101	nd	nd	0.9	RI, MS
15	Nonanal	1104	nd	nd	**5.4**	RI, MS
16	*α*-Campholenal	1127	1.0	nd	nd	RI, MS
17	*trans*-Pinocarveol	1141	**3.0**	nd	nd	RI, MS
18	3-Nonen-2-one	1143	nd	nd	1.9	RI, MS
19	Camphor	1145	1.7	nd	nd	RI, MS
20	Camphene hydrate	1150	0.9	nd	nd	RI, MS
21	*p*-Vinylanisole	1154	nd	nd	1.0	RI, MS
22	*(E)*-2-Nonenal	1163	nd	nd	0.9	RI, MS
23	Pinocarvone	1164	1.4	nd	nd	RI, MS
24	Borneol	1167	**12.5**	nd	0.9	RI, MS
25	4-Terpineol	1179	**5.7**	nd	0.9	RI, MS
26	Myrtenol	1195	1.3	nd	nd	RI, MS
27	*β*-Methyl-*γ*-octalactone	1196	nd	nd	0.8	RI, MS
28	Safranal	1197	nd	nd	1.1	RI, MS
29	*β*-Cyclocitral	1222	nd	nd	1.5	RI, MS
30	*β*-Cyclohomocitral	1256	nd	nd	1.0	RI, MS
31	*(E)*-2-Decenal	1263	nd	nd	1.2	RI, MS
32	Nonanoic acid	1281	nd	nd	1.4	RI, MS
33	Carvacrol	1301	nd	nd	0.9	RI, MS
34	Decahydro-1,5-dimethyl naphthalene	1305	nd	nd	1.6	RI, MS

No.[a]	Compounds	RI[b]	Essential Oils (%)[c]			Identification[f]
			C. ladaniferus	*C. albidus*	*C. monspeliensis*	
35	Theaspirane II	1315	nd	nd	0.9	RI, MS
36	*(Z)*-3-Hexenyl 2-methylcrotonate	1324	nd	nd	1.4	RI, MS
37	*α*-Longipinene	1352	nd	1.2	nd	RI, MS
38	*α*-Cubebene	1352	1.0	nd	nd	RI, MS
39	Longicyclene	1373	1.2	nd	nd	RI, MS
40	*α*-Copaene	1377	nd	1.0	1.2	RI, MS
41	*β*-Bourbonene	1385	nd	**8.7**	nd	RI, MS
42	*β*-Cubebene	1391	1.0	0.8	nd	RI, MS
43	*iso*-Italicene	1399	nd	0.5	nd	RI, MS
44	*trans*-*α*-Ambrinol	1414	nd	nd	1.5	RI, MS
45	*β*-Caryophyllene	1419	nd	**4.5**	1,0	RI, MS
46	7,8-Dihydro-3,4-dehydro-*β*-ionone	1424	nd	nd	1.4	RI, MS
47	*(E)*-*α*-Ionone	1428	nd	nd	**3.0**	RI, MS
48	*β*-Copaene	1430	nd	1.4	nd	RI, MS
49	*cis*-*α*-Ambrinol	1437	nd	nd	**3.3**	RI, MS
50	*α*-Guaiene	1440	nd	**2.4**	nd	RI, MS
51	*α*-Humulene	1456	nd	1.5	nd	RI, MS
52	*Allo*-Aromadendrene	1462	nd	1.0	nd	RI, MS
53	*γ*-Gurjunene	1474	nd	nd	1.5	RI, MS
54	*trans*-Cadina-1(6),4-diene	1475	1.0	nd	nd	RI, MS
55	*γ*-Muurolene	1479	nd	**5.6**	nd	RI, MS
56	*ar*-Curcumene	1483	nd	**8.3**	nd	RI, MS
57	*(E)*-*β*-Ionone	1487	nd	nd	1.6	RI, MS
58	*trans*-Muurola-4(14),5-diene	1493	0.8	nd	nd	RI, MS
59	*epi*-Cubebol	1494	1.6	nd	nd	RI, MS
60	*α*-Zingiberene	1496	nd	**7.4**	nd	RI, MS
61	*trans*-*γ*-Cadinene	1514	nd	1.2	nd	RI, MS
62	Cubebol	1515	1.2	nd	nd	RI, MS
63	*δ*-Cadinene	1524	**7.6**	**3.1**	nd	RI, MS
64	8,14-Cedranoxide	1540	nd	0.6	nd	RI, MS
65	*(Z)*-3-Hexenyl benzoate	1570	nd	nd	**2.8**	RI, MS
66	Spathulenol	1577	nd	1.3	nd	RI, MS
67	*ar*-Turmerol	1581	nd	**2.9**	nd	RI, MS
68	Caryophyllene oxide	1582	1.6	**2.8**	nd	RI, MS
69	Globulol	1584	nd	1.8	nd	RI, MS

No.[a]	Compounds	RI[b]	Essential Oils (%)[c]			Identification[f]
			C. ladaniferus	*C. albidus*	*C. monspeliensis*	
70	Gleenol	1586	0.7	nd	nd	RI, MS
71	Viridiflorol	1591	**6.4**	nd	0.9	RI, MS
72	*cis*-Arteannuic alcohol	1595	0.7	nd	nd	RI, MS
73	5,7-di-*epi*-α-Eudesmol	1603	**13.6**	nd	nd	RI, MS
74	Humulene epoxide II	1607	nd	0.5	nd	RI, MS
75	1,10-di-*epi*-Cubenol	1615	nd	**4.4**	nd	RI, MS
76	1-*epi*-Cubenol	1628	**2.6**	**2.9**	nd	RI, MS
77	T-Cadinol	1641	nd	**4.0**	nd	RI, MS
78	Cubenol	1642	1.6	nd	nd	RI, MS
79	T-Muurolol	1642	nd	2.5	nd	RI, MS
80	α-Eudesmol	1654	nd	2.2	nd	RI, MS
81	*cis*-α-Santalol	1682	nd	0.6	nd	RI, MS
82	*epi*-α-Bisabolol	1686	nd	**11.4**	nd	RI, MS
83	*ar*-Curcumen-15-al	1710	nd	1.2	nd	RI, MS
84	Xanthorrhizol	1753	nd	0.5	nd	RI, MS
85	14-Hydroxy-α-muurolene	1772	nd	**5.2**	nd	RI, MS
86	Hexahydrofarnesylacetone	1845	nd	nd	1.4	RI, MS
87	*(E)*-Nuciferyl acetate	1879	nd	nd	1.5	RI, MS
88	Kaur-15-ene	1988	nd	nd	1.8	RI, MS
89	Phyllocladene	2013	nd	nd	1.5	RI, MS
90	*epi*-13-Manoyl oxide	2011	nd	nd	**28.6**	RI, MS
91	Kaur-16-ene	2040	nd	nd	**8.1**	RI, MS
	Total Identification %		**99.6**	**93.4**	**90.3**	
	Yields % (w/w)		0.080%	0.020%	0.003%	
	Monoterpene hydrocarbons		**25.6**	0.0	0.0	
	Oxygenated monoterpenes		**27.5**	0.0	3.6	
	Sesquiterpene hydrocarbons		12.6	**48.6**	3.7	
	Oxygenated sesquiterpenes		**30.0**	**44.8**	2.4	
	Diterpene hydrocarbons		0.0	0.0	11.4	
	Oxygenated diterpenes		0.0	0.0	**28.6**	
	Apocarotenes		0.0	0.0	16.7	
	Non-terpene derivatives		3.9	0.0	**23.9**	

The main compounds are reported in bold.
[a] Order of elution is given on non-polar capillary column DB-5. [b] Retention indices (RI) were determined relatively to the retention time of a series of *n*-alkanes. [c] Relative percentage calculated by GC-FID on DB-5. [d] nd: not detected. [f] RI: Retention Indices; MS: Mass Spectrum.

Table 2. Antimicrobial Activity of Essential Oils of *C. ladaniferus*, *C. albidus* and *C. monspeliensis*

Microorganisms		Gram-positive				Gram-negative			Yeast	
		B. subtilis ATCC 6633	*S. aureus* ATCC 25923	*L. monocytogenes* ATCC 15313	*E. fæcalis* ATCC 49452	*K. pneumoniae* ATCC 70603	*E. coli* ATCC25912	*C. freundii* ATCC 8090	*C. albicans* ATCC 10231	*C. albicans* ATCC 26790
Essential oils[a]		**DI[b] (mm)**								
CL	5 µL	11±0.577	12±0.288	8±0.288	-[e]	-	-	-	-	-
	10 µL	14±0	12±0.288	8±0.288	-	-	-	-	-	-
	15 µL	14±0.288	12±0.288	9±0.288	8±0.577	-	-	-	-	-
	20 µL	15±0.288	12±0.288	10±0.288	8±0.577	-	-	-	-	-
	25 µL	15±0	12±0	12±0.288	8±0.577	-	-	-	-	-
	30 µL	15±0	12±0	12±0.288	8±0.577	8±0.577	-	-	-	-
CA	5 µL	15±0	12±0.288	9±0.288	-	-	-	-	8±0.288	-
	10 µL	15±0	12±0.288	11±0.577	-	-	-	-	8±0.288	-
	15 µL	15±0	12±0	11.666±0,33	-	8±0.577	-	-	8±0.288	-
	20 µL	15±0	12±0	11.666±0.33	-	10.333±0.881	-	-	8±0.288	-
	25 µL	16±0.288	12±0	11.666±0.33	-	10.333±0.881	-	-	10±0.288	-
	30 µL	16±0.288	12±0.288	11.666±0.33	-	10.333±0.881	-	-	13±0.288	-
CM	5 µL	12±0.288	14±0	-	-	8±0.5773503	-	8±0.288	-	-
	10 µL	13±0.288	15±0	-	-	10.333±0.881	-	12±0.288	-	-
	15 µL	19±0.577	16±0	-	-	13±0.577	-	12±0.288	-	-
	20 µL	20±0.577	20±0.288	-	-	14±0.577	-	12±0.288	-	-
	25 µL	20±0.577	25±0.288	-	-	14±0	-	15±0.288	-	-
	30 µL	23±0.288	25±0.288	-	-	14±0	-	15±0	-	-
Antibiotics[c]		DI[b] (mm)								
GENT	10 µg	22±0	25±0	12±0	13±0	15±0	23±0	32±0	-	-
AmB	100 µg	-	-	-	-	-	-	-	32±0	30±0
		MIC[d] (µg/mL)								
CL		10±0.000	20±0.000	20±0.000	20±0.000	-	-	-	-	-
CA		20±0.000	20±0.000	20±0.000	-	20±0.000	-	-	20±0.000	-
CM		0.25±0.000	0.25±0.000	-	-	20±0.000	-	-	-	-
GENT	10 µg	5.2±0.000	0.19±0.000	2.21±0.000	0.78±0.000	4.16±0.000	0.32±0.000	0.19±0.000	-	-
AmB	100 µg	-	-	-	-	-	-	-	8±0.000	4±0.000

Values were expressed as mean±SD (n=3) using PAST3.03 program. [a] Essential oils: CL: *Cistus ladaniferus*; CA: *Cistus albidus*; CM: *Cistus monspeliensis*; [b] DI: Diameter of inhibition zone in mm including the disc diameter (6mm); [c] Antibiotics: GENT: Gentamicin; AmB: Amphotericin B; [d] MIC: Minimum inhibitory concentration expressed in µg/mL. [e] -: no inhibition zone; ATCC: American Type Culture Collection.

These studies report a clear prevalence of sesquiterpene hydrocarbons, with α-zingiberene as the main one, reaching 8.1-20.7% in the essential oil extracted from flowers (Palá-Paúl et al., 2005; Macionni et al., 2007) and 12.8% in that of the aerial parts (Paolini et al., 2008). Oxygenated sesquiterpenes are reported in lesser percentages; generally well below those of sesquiterpene hydrocarbons. The most representative are *epi*-α-bisabolol (6.6%) and globulol (2.4%) in the leaves (Palá-Paúl et al., 2005; Macionni et al., 2007) and caryophyllene oxide (3.8%) in the aerial parts (Paolini et al., 2008). Therefore, the essential oil obtained from the aerial parts of *C. albidus* growing in the North-West of Algeria is clearly different from the one cited above because of the relevant presence of sesquiterpene derivatives, in a percentage (48.6%) similar to the oxygenated ones (44.8%). β-Bourbonene (8.7%) and *epi*-α-bisabolol (11.4%) were identified as the main compounds among hydrocarbons and oxygenated sesquiterpenes, respectively.

It should also be noted that previous studies on the composition of the essential oil of *C. monspeliensis* from different regions of the Mediterranean basin are present in literature with very variable results. At first glance, the diterpenes *epi*-13-manoyl oxide, kaur-16-ene and the oxygenated sesquiterpene caryophyllene oxide have been identified as the major compounds in the Greek oil isolated from the leaves (Angelopoulou et al., 2001), but in substantial different percentages (39.69, 18.51 and 8.50%, respectively) with respect to the present one from Algeria. Notably, caryophyllene oxide was completely absent in our oil. The apocarotenes and non-terpene compounds are present in quite low percentages *(E)*-α-ionone (0.87%), nonanal (0.68%). On the other hand, the essential oils isolated from fruits are characterized by the presence of kaur-16-ene (3.58%) (Angelopoulou et al., 2001). In another report, Angelopolou et al. (2002) analyzed the diurnal and the seasonal variation of the essential oil from leaves. They noted that in their oil the manoyl oxide mixture of isomers (*epi-13*-manoyl oxide and *diepi*-8,13-manoyl oxide) reached 27.07% (Angelopoulou et al., 2002); the latter derivative was not identified in our sample. The Tunisian oil is also characterized by the predominance of the *epi-13*-manyol oxide (17.7%), borneol (6.3%) and kaur-16-ene (3.6%) (Loizzo et al., 2013). On the contrary, borneol is found in our Algerian oil in much smaller amounts (0.9%). In Morocco, Oller-Lopez et al. (2005) identified the oxygenated diterpenoid *epi-13*-manoyl oxide as the main component of essential oils from leaves, whereas no oxygenated diterpenes have been detected in the study of Martos et al. (2011). However, α-pinene (5.6%) and sabinene (0.7%) are present in the monoterpene hydrocarbons fraction of the oil, whilst the Algerian essential oil does not contain monoterpene hydrocarbons at all. Apocarotenes were identified in smaller amount (7.8%) with respect to our oil (16.7%). In 1984, Gülz investigated essential oils from leaves and stems of the plant, evidencing the low amounts of mono- and sesquiterpene hydrocarbons. He hypothesized the presence of diterpene as the main component (more than 40%), but he was not able to identify its exact chemical structure.

The essential oils of studied Cistus displayed a variable degree of antimicrobial activity with *C. monspeliensis* oil showing an important antimicrobial effectiveness if compared with the activity of the other oils. In general, Gram-positive bacteria were found to be more sensitive to the essential oils than Gram-negative bacteria. Indeed, at a dose of 30 µL/disk, *C. monspeliensis* oil was active on Gram-positives bacteria: *Bacillus subtilis* (23±0.288 mm), *Staphylococcus aureus* (25±0.288 mm) and on Gram-negatives strains: *Klebsiella pneumoniae* (14±0 mm), *Citrobacter freundii* (15±0 mm). Essential oils from *C. ladaniferus* and *C. albidus* were active on both *Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 25923 bacteria, while an average activity was observed towards *Listeria monocytogenes* ATCC 15313. On the other hand, a low activity was registered against *Enterococcus faecalis* ATCC 49452 and *Klebsiella pneumoniae* ATCC 70603 for *C. ladaniferus* oil and a moderate activity towards *Klebsiella pneumoniae* ATCC 70603 for *C. albidus* oil. No inhibition zone was observed for other strains. Compared to the reference antibiotics, the differences are significant for all the strains, except for *Staphylococcus aureus* ATCC 25923 where the inhibition of *C. monspeliensis* volatiles (25 and 30 µL/disk) against *Staphylococcus* strains is the same caused by gentamicin (25±0.288 mm in diameter). The same was observed towards *Listeria monocytogenes* ATCC 15313 for the essential oils of *C. ladaniferus* (25 and 30 µL/disk) and *C. albidus* (from 5 and 10 µL/disk), with an equal inhibition diameter of 12±0.288 mm.

The MICs values of *C. ladaniferus* and *C. albidus* oils against *Bacillus subtilis* ATCC 6633 were 10 µg/L and 20 µg/L, respectively. An interesting MIC (0.25±0 µg/mL) was registered for *C. monspeliensis* oils, a value better than gentamicin against the same pathogen (5.2±0 µg/mL).

Similar findings were observed for *Staphylococcus strains*, with a MIC of 0.25±0 µg/L for *C. monspeliensis* oil, almost identical to gentamicin (0.19±0 µg/L). The other oils presented higher values (20±0 µg/L). Also towards other strains, *Listeria monocytogenes* ATCC 15313, *Enterococcus faecalis* ATCC 49452 and *Klebsiella pneumoniae* ATCC 70603, the MIC values were 20±0 µg/L.

Only the essential oil of *C. albidus* was active against the yeasts, in particular on *Candida albicans* ATCC 10231 (MIC about 20±0 µg/L). However, the difference was very significant with respect to the reference drug amphotericin B. This behavior is probably due to the multi-resistance that characterizes these two yeasts strains. The antimicrobial activity of essential oils is obviously dependent on its chemical composition. Oxygenated compounds are generally more effective than hydrocarbons. In particular, among the most active components, phenol derivatives should be mentioned, together with the less active alcohols (Kalemba and Kunicka, 2003).

Conclusion

The current study presents, for the first time, the results of the chemical composition of the essential oils from three species of *Cistus* growing in the North-West of Algeria. The composition of the essential oils from the aerial parts of *C. ladaniferus*, *C. albidus* and *C. monspeliensis* was quite different. On the other side, qualitative and quantitative differences in the composition of the oils have been shown with those from literature. This variation is attributed to many factors: climate and soil conditions, part studied of the plant, fresh or dry material, moment of collection and method of extraction of the oil. The results of the antimicrobial activity showed that the essential oils of the three *Cistus* spices present new antimicrobial agents to treat infections, so they must be more investigated.

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Author Contribution Statement

A. H. designed research; **B. K.**, **B. H.**, **A. N.** and **F. G.** performed the research and analyzed the data; A. H. wrote the article. All authors read and approved the final manuscript.

Conflict of interest

The authors declare that there are no conflicts of interest.

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