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 (Aranghi 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-Palú 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 (Barragán-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. salvifolius, 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-Palú et al., 2005; Zidane et al., 2013; Zidane et al., 2013; 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°5’60” 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.
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 Clevenger’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.

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

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 (10^6 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 monoterpenes 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*-a-eudesmol (13.6%), borneol (12.5%), camphe (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-*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 monoterpane 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, monoterpane 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., 2013; 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 13.9% 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

<table>
<thead>
<tr>
<th>No.</th>
<th>Compounds</th>
<th>RI bent</th>
<th>Essential Oils (%)</th>
<th>Identification&lt;sup&gt;f&lt;/sup&gt;</th>
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</thead>
<tbody>
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<td></td>
<td></td>
<td>RI&lt;sup&gt;a&lt;/sup&gt;</td>
<td><em>C. ladaniferus</em></td>
<td><em>C. albidus</em></td>
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<tr>
<td>1</td>
<td>(E)-3-Hexen-1-ol</td>
<td>853</td>
<td>nd&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
<td>2</td>
<td>Tricyclene</td>
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<td>α-Pinene</td>
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<td>Sabinene</td>
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<td>6-Methyl-5-hepten-2-one</td>
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<td>2-Methyl-2-hepten-4-one</td>
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<td>α-Terpineene</td>
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<td>9</td>
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<td>10</td>
<td>Cistus cyclohexanone (syn. 2,2,6-Trimethyl cyclohexanone)</td>
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<td>Essential Oils (%)&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>C. ladaniferus</td>
<td>C. albids</td>
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<td>trans-Muurola-4(14),5-diene</td>
<td>1493</td>
<td>0.8</td>
<td>nd</td>
</tr>
<tr>
<td>59</td>
<td>epι-Cubebol</td>
<td>1494</td>
<td>1.6</td>
<td>nd</td>
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<tr>
<td>60</td>
<td>a-Zingiberene</td>
<td>1496</td>
<td>nd</td>
<td>7.4</td>
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<td>trans-γ-Cadinene</td>
<td>1514</td>
<td>nd</td>
<td>1.2</td>
</tr>
<tr>
<td>62</td>
<td>Cubebol</td>
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<td>nd</td>
</tr>
<tr>
<td>63</td>
<td>δ-Cadinene</td>
<td>1524</td>
<td>7.6</td>
<td>3.1</td>
</tr>
<tr>
<td>64</td>
<td>8,14-Cedranoxide</td>
<td>1540</td>
<td>nd</td>
<td>0.6</td>
</tr>
<tr>
<td>65</td>
<td>(Z)-3-Hexenyl benzoate</td>
<td>1570</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>66</td>
<td>Spathulenol</td>
<td>1577</td>
<td>nd</td>
<td>1.3</td>
</tr>
<tr>
<td>67</td>
<td>ar-Turmerol</td>
<td>1581</td>
<td>nd</td>
<td>2.9</td>
</tr>
<tr>
<td>68</td>
<td>Caryophyllene oxide</td>
<td>1582</td>
<td>1.6</td>
<td>2.8</td>
</tr>
<tr>
<td>69</td>
<td>Globulol</td>
<td>1584</td>
<td>nd</td>
<td>1.8</td>
</tr>
</tbody>
</table>
Chemical Analysis of the Essential Oils of Three *Cistus* Species Growing in North-West of Algeria

<table>
<thead>
<tr>
<th>No.</th>
<th>Compounds</th>
<th>RI</th>
<th>Essential Oils (%)</th>
<th>Identification</th>
</tr>
</thead>
<tbody>
<tr>
<td>70</td>
<td>Glennol</td>
<td>1586</td>
<td>0.7</td>
<td>nd</td>
</tr>
<tr>
<td>71</td>
<td>Viridiflorol</td>
<td>1591</td>
<td><strong>6.4</strong></td>
<td>nd</td>
</tr>
<tr>
<td>72</td>
<td><em>cis</em>-Arteannuic alcohol</td>
<td>1595</td>
<td>0.7</td>
<td>nd</td>
</tr>
<tr>
<td>73</td>
<td>5,7-di-epi-α-Eudesmol</td>
<td>1603</td>
<td><strong>13.6</strong></td>
<td>nd</td>
</tr>
<tr>
<td>74</td>
<td>Humulene epoxide II</td>
<td>1607</td>
<td>nd</td>
<td>0.5</td>
</tr>
<tr>
<td>75</td>
<td>1,10-di-epi-Cubenol</td>
<td>1615</td>
<td>nd</td>
<td><strong>4.4</strong></td>
</tr>
<tr>
<td>76</td>
<td>1-epi-Cubenol</td>
<td>1628</td>
<td><strong>2.6</strong></td>
<td><strong>2.9</strong></td>
</tr>
<tr>
<td>77</td>
<td>T-Cadinol</td>
<td>1641</td>
<td>nd</td>
<td><strong>4.0</strong></td>
</tr>
<tr>
<td>78</td>
<td>Cubenol</td>
<td>1642</td>
<td>1.6</td>
<td>nd</td>
</tr>
<tr>
<td>79</td>
<td>T-Muurolole</td>
<td>1642</td>
<td>nd</td>
<td>2.5</td>
</tr>
<tr>
<td>80</td>
<td>α-Eudesmol</td>
<td>1654</td>
<td>nd</td>
<td>2.2</td>
</tr>
<tr>
<td>81</td>
<td><em>cis</em>-α-Santalol</td>
<td>1682</td>
<td>nd</td>
<td>0.6</td>
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<tr>
<td>82</td>
<td>epi-α-Bisabolol</td>
<td>1686</td>
<td>nd</td>
<td><strong>11.4</strong></td>
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<tr>
<td>83</td>
<td><em>ar</em>-Curcumen-15-al</td>
<td>1710</td>
<td>nd</td>
<td>1.2</td>
</tr>
<tr>
<td>84</td>
<td>Xanthorrhizol</td>
<td>1753</td>
<td>nd</td>
<td>0.5</td>
</tr>
<tr>
<td>85</td>
<td>14-Hydroxy-α-murolene</td>
<td>1772</td>
<td>nd</td>
<td><strong>5.2</strong></td>
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<tr>
<td>86</td>
<td>Hexahydrofarnesylacetone</td>
<td>1845</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>87</td>
<td>(E)-Nuciferyl acetate</td>
<td>1879</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>88</td>
<td>Kaur-15-ene</td>
<td>1988</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>89</td>
<td>Phyllocladene</td>
<td>2013</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>90</td>
<td>epi-13-Manoyl oxide</td>
<td>2011</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>91</td>
<td>Kaur-16-ene</td>
<td>2040</td>
<td>nd</td>
<td>nd</td>
</tr>
</tbody>
</table>

| 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.

*Order of elution is given on non-polar capillary column DB-5. Retention indices (RI) were determined relatively to the retention time of a series of *n*-alkanes. Relative percentage calculated by GC-FID on DB-5. nd: not detected. RI: Retention Indices; MS: Mass Spectrum.*
### Table 2. Antimicrobial Activity of Essential Oils of *C. ladaniferus*, *C. albidus* and *C. monspeliensis*

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Gram-positive</th>
<th>Gram-negative</th>
<th>Yeast</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>B. subtilis</em> ATCC 6633</td>
<td><em>S. aureus</em> ATCC 25923</td>
<td><em>L. monocytogenes</em> ATCC 15313</td>
</tr>
<tr>
<td>Essential oils</td>
<td>CL: <em>Cistus ladaniferus</em>; CA: <em>Cistus albidus</em>; CM: <em>Cistus monspeliensis</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Essential oilsa</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 µL</td>
<td>11±0.577</td>
<td>12±0.288</td>
<td>8±0.288</td>
</tr>
<tr>
<td>10 µL</td>
<td>14±0</td>
<td>12±0.288</td>
<td>8±0.288</td>
</tr>
<tr>
<td>15 µL</td>
<td>14±0.288</td>
<td>12±0.288</td>
<td>9±0.288</td>
</tr>
<tr>
<td>20 µL</td>
<td>15±0.288</td>
<td>12±0.288</td>
<td>10±0.288</td>
</tr>
<tr>
<td>25 µL</td>
<td>15±0</td>
<td>12±0</td>
<td>12±0.288</td>
</tr>
<tr>
<td>30 µL</td>
<td>15±0</td>
<td>12±0</td>
<td>12±0.288</td>
</tr>
<tr>
<td>5 µL</td>
<td>15±0</td>
<td>12±0.288</td>
<td>9±0.288</td>
</tr>
<tr>
<td>10 µL</td>
<td>15±0</td>
<td>12±0.288</td>
<td>11±0.577</td>
</tr>
<tr>
<td>15 µL</td>
<td>15±0</td>
<td>12±0</td>
<td>11.666±0.33</td>
</tr>
<tr>
<td>CA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 µL</td>
<td>15±0</td>
<td>12±0</td>
<td>11.666±0.33</td>
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<tr>
<td>25 µL</td>
<td>16±0.288</td>
<td>12±0</td>
<td>11.666±0.33</td>
</tr>
<tr>
<td>30 µL</td>
<td>16±0.288</td>
<td>12±0.288</td>
<td>11.666±0.33</td>
</tr>
<tr>
<td>CM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 µL</td>
<td>12±0.288</td>
<td>14±0</td>
<td>-</td>
</tr>
<tr>
<td>10 µL</td>
<td>13±0.288</td>
<td>15±0</td>
<td>-</td>
</tr>
<tr>
<td>15 µL</td>
<td>19±0.577</td>
<td>16±0</td>
<td>-</td>
</tr>
<tr>
<td>Antibiotics</td>
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<tr>
<td>GENT</td>
<td>10 µg</td>
<td>22±0</td>
<td>25±0</td>
</tr>
<tr>
<td>AmB</td>
<td>100 µg</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MIC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL</td>
<td>10±0.000</td>
<td>20±0.000</td>
<td>20±0.000</td>
</tr>
<tr>
<td>CA</td>
<td>20±0.000</td>
<td>20±0.000</td>
<td>20±0.000</td>
</tr>
<tr>
<td>CM</td>
<td>0.25±0.000</td>
<td>0.25±0.000</td>
<td>-</td>
</tr>
<tr>
<td>GENT</td>
<td>10 µg</td>
<td>5.2±0.000</td>
<td>0.19±0.000</td>
</tr>
<tr>
<td>AmB</td>
<td>100 µg</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Values were expressed as mean±SD (n=3) using PAST3.03 program. * 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, kaurene-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 kaurene-16-ene (3.58%) (Angelopoulou et al., 2001). In another report, Angelopoulou 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-manoxy 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 sabine (0.7%) are present in the monoterpene hydrocarbons fraction of the oil, whilst the Algerian essential oil does not contain monoterpene hydrocarbons of 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 diterpen 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 monocyctogenes 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 species 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.

References


European Committee for Antimicrobial Susceptibility Testing (EUCAST) of the European Society of Clinical Microbiology and Infectious Diseases (Eucmid) (2003).


ACS
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