Assessment of Antioxidant and Antimicrobial Compounds of Volatiles from Leaves, Stems and Flowers of Olives

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Abstract

Protection of olive cultivars, Olea europaea L., from diseases and the development of more sophisticated control methods are indispensable for a renovated and competitive olive sector. In this context, the volatiles obtained by the main Tunisian oil cultivar Chemlali and both the introduced cultivars Arbequina and Koroneiki were tested for their antimicrobial activity against several dangerous pathogens by diffusion and dilution methods (in 2014). To evaluate the adaptation to biotic stress, the antioxidant potential was additionally evaluated. The volatiles extracted from leaves, stems and flowers of the tested cultivars exhibited interesting antimicrobial and antioxidant activities, reaching in many cases 100% of inhibition. To identify the bioactive compounds, GC-FID and GC-MS were performed, permitting to identify up to 97.8% of total compounds. Both non-terpene hydrocarbons and terpenes were present in important proportions among volatiles.

Keywords: olive cultivars, antimicrobial activity, antioxidant activity, non-terpene hydrocarbons, terpenes

Introduction

Tunisia is the main olive producing country in the southern Mediterranean. 34% of its cultivated land is devoted to olive growing, which extends from the north to the south of the country. This sector plays, economic, social and environmental roles, contributing to food security, job creation, equilibrium of the commercial balance, preservation of natural resources and limitation of the rural exodus. The olive forest is dominated by the oil cultivars, Chemlali in the center and south of the country, and Chetoui in the north. Chemlali alone occupies 56% of the olive-growing area and represents 69% of the total number of olive trees.
Other varieties have been introduced in the Tunisian olive system, such as Arbequina and Koroneiki, of Spanish and Greek origin respectively, to improve the productivity and to mitigate the fluctuation problem that characterizes the local varieties. To maintain satisfying productivity and defend the Tunisian position in the world, the olive tree must be well protected from microbes that may have adverse effects on final yield. Nevertheless in the Mediterranean region, olive production is affected by several diseases, Verticillium wilt, caused by *Verticillium dahlia* Kleb., is currently the most devastating disease correlated with low yield and high rates of olive tree loss [2]. *Fusarium solani* causes leaf drop, wilt, and mortality of the olive tree [3]. *Pseudomonas* is a very dangerous bacterium, *Pseudomonas savastanoi* and its pathovars *savastanoi, fraxini,* and *nerii* provoke a disease characterized by tumorous out growths [4]. The contamination of olive tree by *P. savastanoi* pv. *savastanoi* causes to hypertrophy of the stems and branches and, rarely, of the leaves and fruits [5]. Similarly, *Agrobacterium tumefaciens* leads crown gall disease on various plant species, especially olive cultivars, by introducing its T-DNA into the host genome, causing its proliferation and consequently plant tumors. The cited microbes, in the company of many others, have great economic consequences. Since no effective bactericides or fungicides exist, biological control using the naturally occurring antagonistic potential against pathogens is a potentially viable and environmentally friendly alternative [6].

Thus, the aim of the present study is to evaluate the behavior of the principal olive cultivar Chemlali and both the introduced cultivars Arbequina and Koroneiki against many dangerous pathogenic germs and to evaluate their antioxidant capacity to scavenge radicals that could be a consequence of such biotic stress.

**Experimental**

**Plant Material**

Chemlali, Koroneiki and Arbequina, 35 years old, were cultivated in intensive mode (6x6), in “Menzel El Mhiri”, located in Kairouan governorate and Nasrallah delegation (35°21’ North 9°49’ East). From each cultivar, fresh leaves, flowers and stems were harvested during the flowering stage, in 2014.

**Volatile Extraction and Analyses**

Volatile compounds were extracted from the aerial parts of the different cultivars. Fresh leaves, flowers and stems were weighted and crushed and submitted to steam distillation. Obtained samples were conserved at -16°C until tests. The analyses of volatile compounds were performed with GC- FID and GC-MS systems, according to Saidana et al. [7].

**Antimicrobial Activities**

The bacterial strains investigated were: *Pseudomonas savastanoi* pv *savastanoi* EW2009; *Agrobacterium tumefaciens* C58; *Pseudomonas aureofaciens* NCPPB 3335, *Burkholderia glathei* MB196942, *Botrytis cinerea* TAX: 40559, *Fusarium solani* (Mar.) Sac. 1881, *Penicilliu mitalicum* MB162660, *Fusarium oxysporum* f. sp. *Lycopersici* MB 416243. The inhibition zones, MIC, MBC and MFC were determined according to Saidana et al. [7, 8].

**Antioxidant Activity**

*DPPH* and ABTS** scavenging activities were performed according to Saidana et al. [9, 10].

**Statistical Analysis**

Statistical comparisons of the different parameters were performed with SPSS version 20. Analyses of one-way ANOVA, were followed by means comparisons (P = 0.05) and Tukey test.

**Results**

**Volatile Content**

Volatile yield in Chemlali, Koroneiki and Arbequina leaves, stems and flowers varied significantly from 0.01 to 0.024%.

The highest yield of essential oils was found in the flowers of all the cultivars. Compared to the other flowers, those of Chemlali showed the highest yield, reaching 0.024%.

Similarly, Chemlali appeared to be the richest in volatiles in all its organs, followed by Koroneiki then Arbequina. Indeed, the contents of volatiles were 0.019, 0.012 and 0.010% in the leaves, 0.024, 0.017 and 0.015% in the flowers and 0.012, 0.012 and 0.010% in the stems of Chemlali, Koroneiki and Arbequina respectively.

**Antibacterial Activity**

The antibacterial activities of the volatiles extracted from Chemlali, Arbequina, Koroneiki leaves, stems and flowers were tested against both pathogenic strains, *Pseudomonas savastanoi* and *Agrobacterium tumefaciens* and various soil bacteria, such as *Pseudomonas aureofaciens, Burkholderia glathei* and *Bacillus pumilus*.

Leaves of the tested cultivars exhibited an antibacterial activity against *P. savastanoi*, with inhibition diameters of 12, 13 and 13.5 mm for Chemlali, Arbequina and Koroneiki, respectively (Table 1). Flowers exhibited even more interesting
Table 1. Antibacterial activity of volatiles extracted from Chemlali, Arbequina and Koroneiki leaves, stems and flowers against pathogenic strains and soil bacteria.

|          | Pathogenic bacteria | Soil bacteria | Pathogenic fungi |
|----------|---------------------|---------------|------------------|
|          | P.s.  | A.t.  | B.p.  | P.a.  | B.g.  | P. i.  | V.d.  | F.s.  | F. o.  | B. c.  | A.n.  |
| Ch.Le.   | Ø     | 12±0.1\textsuperscript{a} | 10±0.3\textsuperscript{b} | 11±0.2\textsuperscript{b} | 9±0.1\textsuperscript{c} | 9±0.1\textsuperscript{d} | 8±0.3\textsuperscript{b} | 12±0.3\textsuperscript{b} | 8±0.2\textsuperscript{c} | 9±0.1\textsuperscript{b} | - | 9±0.3\textsuperscript{b} |
| MIC      | 125   | 125   | -     | -     | -     | 125   | 125   | 125   | 125   | 250   | 125   |
| MBC/MFC  | >1    | >1    | -     | -     | -     | -     | -     | -     | 1     | -     | -     | 250   |
| Ch.St.   | Ø     | 20±0.1\textsuperscript{a} | 6.5±0.1\textsuperscript{d} | 7±0.3\textsuperscript{c} | 8±0.2\textsuperscript{d} | 10±0.1\textsuperscript{c} | 8±0.3\textsuperscript{b} | 10±0.3\textsuperscript{b} | - | - | 7.5±0.3 | 7.5±0.3\textsuperscript{c} |
| MIC      | 125   | 125   | -     | -     | -     | 125   | 125   | -     | 125   | 250   | 250   |
| MBC/MFC  | >1    | >1    | -     | -     | -     | 250   | -     | -     | -     | -     | -     |
| Ch.Fl.   | Ø     | 14.5±0.3\textsuperscript{b} | 10.5±0.2\textsuperscript{b} | - | 5.5±0.3\textsuperscript{f} | 6±0.1\textsuperscript{f} | 6±0.1\textsuperscript{c} | 15±0.1\textsuperscript{b} | 8±0.3\textsuperscript{c} | 8±0.2\textsuperscript{c} | - | 8±0.1\textsuperscript{c} |
| MIC      | 225   | 225   | -     | -     | -     | -     | -     | -     | 125   | 250   | 125   |
| MBC/MFC  | >1    | >1    | -     | -     | -     | 250   | -     | -     | -     | -     | -     |
| Ar.Le.   | Ø     | 13±0.1\textsuperscript{c} | 9±0.3\textsuperscript{c} | 9±0.1\textsuperscript{c} | 13±0.2\textsuperscript{a} | 10.5±0.2\textsuperscript{b} | 9±0.2\textsuperscript{a} | - | 8±0.3\textsuperscript{c} | 9±0.3\textsuperscript{b} | - | 8±0.3\textsuperscript{c} |
| MIC      | -     | -     | -     | -     | -     | 125   | 125   | 125   | 125   | 250   | 125   |
| MBC/MFC  | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     |
| Ar.St.   | Ø     | 10±0.3\textsuperscript{b} | - | - | - | - | 12±0.1\textsuperscript{b} | 10±0.2\textsuperscript{b} | 8±0.3\textsuperscript{b} | - | 7.5±0.3\textsuperscript{c} |
| MIC      | 125   | -     | -     | -     | -     | 250   | 125   | 125   | -     | 250   | 250   |
| MBC/MFC  | >1    | -     | -     | -     | -     | 500   | -     | -     | -     | -     | -     |
| Ar.Fl.   | Ø     | 13±0.3\textsuperscript{c} | - | 11±0.3\textsuperscript{b} | 9±0.1\textsuperscript{c} | 8±0.2\textsuperscript{c} | - | - | 6±0.2\textsuperscript{c} | 8±0.2\textsuperscript{c} | - | - |
| MIC      | -     | -     | -     | -     | -     | 125   | 125   | 125   | -     | 250   | 125   |
| MBC/MFC  | -     | -     | -     | -     | -     | 250   | -     | -     | -     | -     | -     |
| Ko.Le.   | Ø     | 13.5±0.3\textsuperscript{b} | - | 5.5±0.1\textsuperscript{f} | 7±0.1\textsuperscript{c} | 8±0.1\textsuperscript{e} | - | - | - | 8±0.3\textsuperscript{c} | - | 8±0.3\textsuperscript{c} |
| MIC      | -     | -     | -     | -     | -     | -     | -     | - | - | 250   | 250   |
| MBC/MFC  | -     | -     | -     | -     | -     | -     | -     | - | - | - | - |
| Ko.St.   | Ø     | 10.5±0.3\textsuperscript{b} | - | 8±0.1\textsuperscript{d} | 5.5±0.1\textsuperscript{f} | - | - | 10±0.3\textsuperscript{b} | - | - | - | 9±0.3\textsuperscript{b} |
| MIC      | - | 225 | - | - | - | 250 | 125 | - | - | 250 | 125 |
| MBC/MFC  | - | >1 | - | - | - | 500 | - | - | - | - | - |
| Ko.Fl.   | Ø     | 13.5±0.3\textsuperscript{b} | - | 12±0.1\textsuperscript{c} | 9±0.2\textsuperscript{a} | 10±0.1\textsuperscript{c} | - | 11±0.3\textsuperscript{b} | - | 8±0.3\textsuperscript{b} | - | 8±0.3\textsuperscript{c} |
| MIC      | - | 125 | - | - | - | 250 | - | - | 250 | 250 | 250 | 250 |
activity, presenting inhibitions zones of 14.5, 13 and
13.5 mm, respectively.
However, stem volatiles of Chemlali showed
the best antibacterial activity, which corresponded to the
largest inhibition zone, reaching 20 mm. This activity
was similar to that of Ampicillin, the antibacterial
reference drug. On the contrary, volatiles extracted
from stems of Arbequina and Koroneiki presented
the least activity against 
P. savastanoi, with an
inhibition zone diameter of 10.5 mm. The antibacterial
activities of all tested volatiles against 
A. tumefaciens
were feeble.
Only the volatiles extracted from all the organs
of Chemlali and Arbequina leaves presented
diameter inhibition zones varying from 7 to 11 mm
(Table 1). B. pumilus seemed sensitive to flower volatiles
of Arbequina and Koroneiki and leaf volatiles of
Chemlali, with inhibition zone diameters of 11, 12 and
11 mm. These values were similar to that of Ampicillin,
the antibacterial reference drug. P. aureofaciens
was moderately sensitive to Arbequina leaf volatiles. While
B. glathei appeared resistant to all the volatiles.
The antibacterial activities of all the samples much smaller
than that of Ampicillin against P. aureofaciens and
B. glathei, which presented inhibition zones of 30
and 40 mm, respectively. Stem volatiles of the tested
cultivars were inactive against all tested soil bacteria.
All Chemlali volatiles extracted from leaves, flowers
and stems exhibited an interesting antibacterial activity
against 
P. savastanoi and
A. tumefaciens at quite low
concentrations (Table 1). Visible growth inhibitions
of both cited bacteria were performed by leaf and
stem volatiles of Chemlali at 125 µg/ml; whereas
flower volatiles were active against these bacteria at
225 µg/ml. Bactericidal activities were not determined,
being superior to 1.
Arbequina seemed to be active only through its stem
volatiles against 
P. savastanoi, which visible growth
was inhibited at 125 µg/ml.
While, Koroneiki was active against A. tumefaciens
through its flower and stem volatiles, with visible
growth inhibitions at 125 and 225 µg/ml.

Antifungal Activity

The antifungal activity of the three olive cultivars
was tested against six phytopathogenic fungi,
Verticillium dahlia, Botrytis cinerea, Fusarium solani,
Penicillium italicum, Fusarium oxysporum f. sp.
Lycopersici and Aspergillus niger. According to the
results given in Table 1, V. dahlia appeared to be the most
sensitive to volatiles of olive cultivars, especially leaf and
flower volatiles of Chemlali, which presented inhibition
zones of 12 and 15 mm, respectively. Stem volatiles of
Arbequina and flower volatiles of Koroneiki caused
inhibition zones of 12 and 11 mm. Leaf, flower and stem
volatiles of Chemlali and leaf volatiles of Arbequina
exhibited a moderate antifungal activity against
P. italicum, but interestingly this activity exceeded that
Table 2. Antioxidant activities (%) of volatiles extracted from leaves (Le.), stems (St.) and flowers (Fl.) of Chemlali (Ch.), Koroneiki (Ko.) and Arbequina (Ar.) against the radical DPPH at different concentrations (mg/ml).

| Samples | Concentration mg/ml | IC50 |
|---------|---------------------|------|
|         | 0.125   | 0.25 | 0.5  | 1    | 2    | 4    | 8    | 16   |       |
| Ch.Le.  | 27.89 ±0.2a   | 11.22±0.2b   | 18.25±0.6b   | 37.3±1.0b   | 46.8±3.0b   | 53.28±2.5b   | 68.7±4.2b   | 80.15±1.1b | ±0.015 |
| Ch.St.  | 30.95 ±2.1d   | 30.04±0.5c   | 37.3±1.2e   | 57.02±2.1c   | 80.61±3.7c   | 86.28±4.1c   | 89.9±1.2c   | 89.06±3.6c | ±0.0013 |
| Ch.Fl.  | 28.23 ±0.5d   | 24.03±1.2e   | 38.66±3.8f   | 38.88±2.1c   | 52.72±5.1f   | 64.85±0.1c   | 74.71±0.2c   | 77.21±1.9c | ±0.0009 |
| Ar.Le.  | 52.26 ±2.8g   | 56.12±2.3g   | 92.74±0.9a   | 94.33±0.2a   | 92.29±0.4b   | 93.19±0.4g   | 98.54±1.2c   | 98.16±1.2c | ±0.0008 |
| Ar.St.  | 34.46 ±0.1c   | 29.5±0.2e   | 68.48±0.3g   | 79.02±1.1f   | 84.35±0.7f   | 84.7±4.4c   | 85.51±2.7c   | 85.51±2.7c | ±0.0003 |
| Ar.Fl.  | 27.32 ±0.5f   | 27.77±1.5h   | 36.05±2.3h   | 49.09±4.3h   | 63.71±0.2c   | 72.67±0.3e   | 77.66±0.7c   | 77.66±0.7c | ±0.0006 |
| Ko.Le.  | 40.92 ±1.1f   | 29.02±4.0a   | 37.41±0.3c   | 45.01±3.0a   | 71.76±0.9f   | 83.21±0.6g   | 86.8±1.9d   | 86.8±1.9d | ±0.0014 |
| Ko.St.  | 30.83 ±0.1d   | 26.07±4.0a   | 39.11±0.3c   | 53.17±1.3h   | 73.24±2.8d   | 82.32±1.5h   | 85.83±1.5h   | 85.83±1.5h | ±0.0007 |
| Ko.Fl.  | 8.27 ±2.1e    | 25.96±1.9e   | 41.49±1.7e   | 49.77±1.4e   | 6.12±2.1e    | 74.48±3.7e   | 79.93±0.1e   | 81.85±4.4e | ±0.0012 |
| Trolox  | 95.39 ±0.1e   | 95.32±1.2e   | 94.98±1.5e   | 94.45±2.2e   | 100±0.1e     | 100±0.1e     | 100±0.1e     | 100±0.1e | ±0.0014 |

Different letters indicate statistical significance at the p<0.05 level for each concentration.

Assessment of Antioxidant and Antimicrobial...
The variation of the percentage inhibition of volatiles from leaves, stems and flowers of Chemlali, Arbequina and Koroneiki as a function of time and concentration is illustrated in Fig. 1. Volatiles extracted from all the olive tree parts exhibited an interesting scavenging capacity starting from the first five minutes of contact with the radical cation $\text{ABTS}^\bullet$. This capacity increased gradually with contact time. Leaf volatiles of Chemlali, Arbequina, Koroneiki seemed to be very active against $\text{ABTS}^\bullet$, with 87.5, 100 and 94.85% of radicals scavenged at a concentration of 16 mg/ml. Leaf volatiles of Arbequina were the most active ones, scavenging the totality of radicals at only 1.0 mg/ml and in a short time. Chemlali and Koroneiki exhibited a time depending activity, which became more important over time. The inhibitions of 50% of radicals by leaf volatiles were respectively reached after 30 min of contact at the concentrations of 1.81, 0.316 and 0.18 mg/ml for Chemlali, Arbequina and Koroneiki, respectively.

Fig. 1. Antioxidant activities (%) of volatiles extracted from leaves (A1, A2, A3), stems (B1, B2, B3) and flowers (C1, C2, C3) of Chemlali, Arbequina and Koroneiki against the radical cation $\text{ABTS}^\bullet$. 

[Diagram with graphs showing the antioxidant activities]
Koroneiki presented the lowest IC$_{50}$ after 30 min of contact (Table 3). Also stem volatiles of the studied cultivars were very active against ABTS$^+$, especially those of Chemlali, which inhibited almost the totality of radicals (IC$_{50}$ = 0.722 mg/ml). Koroneiki and Arbequina seemed to be active even at lower concentrations, with IC$_{50}$ of 0.513 and 0.580 mg/ml, respectively. Flower volatiles of Chemlali and Arbequina inhibited over 90% of radicals in almost all time intervals; whereas those of Koroneiki had a slightly weaker activity (80% after 30 min). IC$_{50}$ of these cultivars were respectively 0.784, 0.55 and 0.784 mg/ml for Chemlali, Arbequina and Koroneiki, respectively). Aldehydes were particularly detected in flowers (23.3, 18.6 and 14.7%, respectively). Hydrocarbons appeared to be dominant in all the cultivars, especially in the leaves (5.4, 16.0 and 14.1% for Chemlali, Arbequina and Koroneiki, respectively). Aldehydes were particularly produced by stems of Arbequina (10.4%) and Koroneiki (13.5%). Dihydroedulan IIA was the most represented compound in Koroneiki flowers (17.8%) and apocarotene of Arbequina flowers (18.4%); while, dihydroedulan I in Chemlali (12.2%), Arbequina (10.7%) and Koroneiki (14.7%). Dihydroedulan IIA was the most represented apocarotene of Arbequina flowers (18.4%); while, dihydroedulan I in Koroneiki flowers (17.8%) and 

**Table 3. Radical cation scavenging activity of Chemlali (Ch.), Arbequina (Ar.) and Koroneiki (Ko.) volatiles extracted from leaves (Le.), stems (St.) and flowers (fl.), expressed as Trolox equivalent after 30 min of initial mixing and as 50% of inhibition.**

| Samples | Concentration mg/ml |
|---------|---------------------|
| Ko.Le.  | 0.60 ±0.01         |
| Ch.Le.  | 0.59 ±0.01         |
| Ch.St.  | 0.65 ±0.01         |
| Ch.Fl.  | 1.26 ±0.01         |
| Ar.Le.  | 0.95 ±0.01         |
| Ko.St.  | 1.81 ±0.01         |
| Ko.Fl.  | 2.08 ±0.01         |
| Ar.Fl.  | 2.11 ±0.01         |
| Ko.St.  | 2.11 ±0.01         |
| Ko.Fl.  | 2.11 ±0.01         |
| Samples | Concentration mg/ml |
|---------|---------------------|
| Ch.Le.  | 0.60 ±0.01         |
| Ch.St.  | 0.59 ±0.01         |
| Ch.Fl.  | 0.65 ±0.01         |
| Ar.Le.  | 1.26 ±0.01         |
| Ar.St.  | 1.81 ±0.01         |
| Ar.Fl.  | 2.08 ±0.01         |
| Ko.Le.  | 2.11 ±0.01         |
| Ko.St.  | 2.11 ±0.01         |
| Ko.Fl.  | 2.11 ±0.01         |

Different letters indicate statistical significance at the p<0.05 level for each concentration.

### Chemical Composition of Volatiles

The chemical investigation on the volatiles extracted from different organs of Chemlali, Arbequina and Koroneiki cultivars permitted to characterize 95.7, 94.9 and 91.7% of the total compounds in leaves; 80.6, 84.4 and 87.3% in stems and 95.1, 97.8 and 88.6% in flowers, respectively (Table 4).

Hydrocarbons appeared to be dominant in all the cultivars, especially in their leaves (49.0, 44.8 and 30.7% in Chemlali, Arbequina and Koroneiki, respectively). Similarly, terpenes seemed to be the main chemical class in stems and flowers.

Apocarotenones were particularly produced by stems of Chemlali (34.3%) and flowers of Arbequina (55.4%) and Koroneiki (44.9%). Aromatic derivatives were present in relevant amounts in all the cultivars, especially in the leaves (5.4, 16.0 and 14.1% for Chemlali, Arbequina and Koroneiki, respectively). Aldehydes were particularly detected in flowers (23.3, 18.6 and 14.7%, respectively).

The major aliphatic compounds were 1-hexadecene (34.4%, Chemlali leaves), n-pentadecane (13.5%, Arbequina leaves) and n-dodecane (10.4%, Arbequina flowers). Nonanal was the major aldehyde in flowers of Chemlali (12.2%), Arbequina (10.7%) and Koroneiki (14.7%). Dihydroedulan IIA was the most represented apocarotene of Arbequina flowers (18.4%); while, dihydroedulan I in Koroneiki flowers (17.8%) and 

(E)-β-damascenone in Chemlali leaves (16.8%).

(E)-nerolidol and liguloxide were the major oxygenated sesquiterpenes presents in all the cultivar volatiles, reaching the maximum in Koroneiki leaves (13.1%) and Arbequina stems (11%).
Table 4. Chemical composition of volatiles extracted from leaves (Le), stems (St) and flowers (Fl) of Chemlali, Koroneiki and Arbequina.

| Constituents (%)                  | Chemlali | Arbequina | Koroneiki |
|-----------------------------------|----------|-----------|-----------|
| 2-methyloctane                    | 864      |           | 0.6       |
| p-xylene                          | 867      | 0.8       | 0.2       |
| n-nonane                          | 900      | 4.1       | 6.5       |
| 3-ethyl-1,5-octadiene             | 942      | 0.9       | 2.4       |
| 1-ethyl-4-methylbenzene           | 965      |           | 0.9       |
| phenol                            | 985      | 1.7       |           |
| 2-methyldecane                    | 1062     | 1.8       |           |
| linalool                          | 1101     | 6.1       | 3.8       |
| nonanal                           | 1102     | 3.9       | 12.2      |
| camphor                           | 1145     | 1.5       |           |
| methyl nicotinate                 | 1148     |           | 15.6      |
| Decane, 5,6-dimethyl-              | 1155     |           |           |
| Undecane, 2-methyl-               | 1167     | 1.8       |           |
| 2-Decanol                         | 1185     |           |           |
| (Z)-3-hexenyl butyrate            | 1188     | 2.4       | 2.1       |
| α-terpineol                       | 1191     | 3.2       | 21.9      |
| methyl salicylate                 | 1192     |           | 1         |
| n-dodecane                        | 1200     | 4.8       | 2.1       |
| decanal                           | 1205     | 0.4       |           |
| trans-piperitol                   | 1207     | 2.1       |           |
| β-cyclocitral                     | 1222     | 1.4       | 0.2       |
| (E)-2-decenal                     | 1263     | 11.1      | 5.3       |
| nonanoic acid                     | 1275     | 2.8       |           |
| 2,6,11-trimethyltridecane         | 1277     | 3.3       |           |
| dihydroedulan IIA                 | 1285     | 8.8       | 18.4      |
| p-cymen-7-ol                      | 1290     |           | 12.7      |
| dihydroedulan I                   | 1292     | 2.3       | 7.4       |
| theaspirane I                     | 1298     | 4.9       | 13.6      |
| 4-vinylguaiacol                   | 1313     | 1.3       |           |
| theaspirane II                    | 1315     | 2.1       | 3.9       |
| methyl 4-formylbenzoate            | 1370     |           | 16.0      |
| 3-methyltridecane                 | 1373     | 2.2       | 1.1       |
| (E)-β-damascenone                 | 1382     | 16.8      | 3.2       |
| 10-acetyl-3-carene                | 1389     | 5.5       | 2.7       |
| 1-tetradecene                     | 1392     | 3.1       | 5.2       |
| dihydro-γ-ionone                  | 1396     | 7.4       | 10.9      |
| n-tetradecane                     | 1400     | 3.4       | 4.8       |
| (E)-β-damascone                   | 1412     | 4.8       | 1.2       |
| trans-α-ambrinol                  | 1414     | 2.4       | 3.2       |
Discussion

Volatiles from leaves, stems and flowers of Chemlali, Arbequina and Koroneiki cultivars were characterized. Flowers of all the cultivars were the organs that produced most of the volatiles, with yields reaching 0.024, 0.015 and 0.017%, respectively. Generally, Chemlali produced most volatiles, regardless of the tested organ. Chemlali is of Tunisian origin, while Arbequina and Koroneiki cultivars were introduced for reasons of productivities.

The differences observed for these yields could then be influenced by edaphic and climatic conditions [11], differing in different countries [12]. Additionally, the essential oil content and its composition may vary according to the plant part [13]. To test the effect of

Table 4. Continued.

| Compound                  | Chemlali | Arbequina | Koroneiki | Chemlali | Arbequina | Koroneiki | Chemlali | Arbequina | Koroneiki | Chemlali | Arbequina | Koroneiki |
|---------------------------|----------|-----------|-----------|----------|-----------|-----------|----------|-----------|-----------|----------|-----------|-----------|
| dihydro-α-ionone          | 1420     | 3.9       | 5.0       | 1420     | 3.9       | 5.0       | 1420     | 3.9       | 5.0       | 1420     | 3.9       | 5.0       |
| nerylacetone              | 1436     | 8.0       | 9.3       | 1436     | 8.0       | 9.3       | 1436     | 8.0       | 9.3       | 1436     | 8.0       | 9.3       |
| (E)-geranylacetone        | 1456     | 3.4       | 8.0       | 1456     | 3.4       | 8.0       | 1456     | 3.4       | 8.0       | 1456     | 3.4       | 8.0       |
| (E)-β-ionone              | 1487     | 3.5       | 3.4       | 1487     | 3.5       | 3.4       | 1487     | 3.5       | 3.4       | 1487     | 3.5       | 3.4       |
| 1-pentadecene             | 1492     | 1.0       | 2.4       | 1492     | 1.0       | 2.4       | 1492     | 1.0       | 2.4       | 1492     | 1.0       | 2.4       |
| n-pentadecane             | 1500     | 13.5      | 2.3       | 1500     | 13.5      | 2.3       | 1500     | 13.5      | 2.3       | 1500     | 13.5      | 2.3       |
| (E,E)-α-farnesene         | 1507     | 1.6       | 1.6       | 1507     | 1.6       | 1.6       | 1507     | 1.6       | 1.6       | 1507     | 1.6       | 1.6       |
| liguloxide                | 1532     | 9.5       | 11.0      | 1532     | 9.5       | 11.0      | 1532     | 9.5       | 11.0      | 1532     | 9.5       | 11.0      |
| dihydroactinidiolide      | 1536     | 7.8       | 3.5       | 1536     | 7.8       | 3.5       | 1536     | 7.8       | 3.5       | 1536     | 7.8       | 3.5       |
| epi-ligulyl oxide         | 1551     | 2.3       | 2.7       | 1551     | 2.3       | 2.7       | 1551     | 2.3       | 2.7       | 1551     | 2.3       | 2.7       |
| 4-methylpentadecane       | 1556     | 2.2       |           | 1556     | 2.2       |           | 1556     | 2.2       |           | 1556     | 2.2       |           |
| 2-methylpentadecane       | 1563     |           |           | 1563     |           |           | 1563     |           |           | 1563     |           |           |
| (E)-nerolidol             | 1564     | 9.8       | 3.9       | 1564     | 9.8       | 3.9       | 1564     | 9.8       | 3.9       | 1564     | 9.8       | 3.9       |
| (Z)-3-hexenyl benzoate    | 1570     | 5.4       | 11.3      | 1570     | 5.4       | 11.3      | 1570     | 5.4       | 11.3      | 1570     | 5.4       | 11.3      |
| hexyl benzoate            | 1580     |           |           | 1580     |           |           | 1580     |           |           | 1580     |           |           |
| caryophyllene oxide       | 1582     |           | 3.1       | 1582     |           | 3.1       | 1582     |           | 3.1       | 1582     |           | 3.1       |
| 1-hexadecene              | 1593     | 34.3      | 4.9       | 1593     | 34.3      | 4.9       | 1593     | 34.3      | 4.9       | 1593     | 34.3      | 4.9       |
| n-hexadecane              | 1600     | 11.3      | 7.4       | 1600     | 11.3      | 7.4       | 1600     | 11.3      | 7.4       | 1600     | 11.3      | 7.4       |
| Valerianol                | 1656     | 4.4       |           | 1656     | 4.4       |           | 1656     | 4.4       |           | 1656     | 4.4       |           |

Hydrocarbons              | 49.0     | 17.1      | 8.2       | 44.8     | 31.3      | 18.4      | 30.7     | 35.7      |
Monoterpenic hydrocarbons | 5.5      | 2.7       | 1.9       | 5.5      | 2.7       | 1.9       | 5.5      | 2.7       | 1.9       |
Oxygenated monoterpenes   | 10.8     | 25.7      | 2.1       | 10.8     | 25.7      | 2.1       | 10.8     | 25.7      | 2.1       |
Sesquiterpenic hydrocarbons|         |           |           |         |           |           |         |           |           |
Oxygenated sesquiterpenes | 14.2     | 11.8      | 3.9       | 14.2     | 11.8      | 3.9       | 14.2     | 11.8      | 3.9       |
Apocarotenes              | 21.6     | 34.3      | 19.9      | 21.6     | 34.3      | 19.9      | 21.6     | 34.3      | 19.9      |
Terpenes                  | 41.3     | 59.6      | 45.6      | 33.0     | 50.3      | 57.7      | 46.3     | 50.1      | 73.9      |
Aromatic hydrocarbon       | 0.8      |           | 1.1       | 0.8      |           | 1.1       | 0.8      |           | 1.1       |
Aromatic esters           | 5.4      | 12.2      | 13.0      | 5.4      | 12.2      | 13.0      | 5.4      | 12.2      | 13.0      |
Aromatic compounds        | 5.4      | 16.0      | 14.1      | 5.4      | 16.0      | 14.1      | 5.4      | 16.0      | 14.1      |
Aldehydes                 | 3.9      | 23.3      | 11.1      | 3.9      | 23.3      | 11.1      | 3.9      | 23.3      | 11.1      |
Nitrogen compounds        |         |           | 15.6      |         |           | 15.6      |         |           | 15.6      |
Fatty acid and its ester  | 2.4      | 2.8       | 3.1       | 2.4      | 2.8       | 3.1       | 2.4      | 2.8       | 3.1       |
No aromatic compounds     | 3.9      | 41.3      | 1.1       | 2.8      | 21.7      | 0.6       | 2.8      | 21.7      | 0.6       |
Total identified compounds| 95.7     | 80.6      | 95.1      | 94.9     | 84.4      | 97.8      | 91.7     | 87.3      | 88.6      |
volatiles extracted from the different organs of the three cultivars, they were tested against the most pathogenic bacteria for the olive tree, *Pseudomonas savastanoi* and *Agrobacterium tumefaciens*, by both the diffusion and broth dilution methods. Interaction with some soil bacteria, such as *Pseudomonas aureofaciens*, *Burkholderia glathei* and *Bacillus pumilus* were also noted.

*P. savastanoi* seemed to be more susceptible to the applied olive volatiles than *A. tumefaciens*, with inhibition zones varying from 10 to 20 mm. All the volatiles seemed to be active against this bacterium, but Chemlali stem volatiles had the best effect. In the case of *A. tumefaciens*, only volatiles extracted from leaves, flowers and stems of Chemlali and Arbequina leaf volatiles exhibited inhibition zones that reached a maximum of 10.5 mm.

Interaction of olive volatiles with soil bacteria was variable according to the tested microorganism, cultivar and organ. Indeed, *B. pumilus* seemed to be the most sensitive, with inhibition diameters similar to that of Ampicillin.

The other bacteria, *P. aureofaciens* and *B. glathei*, presented much smaller inhibition zones (13 and 10 mm, respectively) than those registered for the antibacterial reference drug (30 and 40 mm, respectively). Chemlali volatiles showed inhibition zones against both bacteria and inhibited their visible growth at quite low concentration (125 µg/ml).

Arbequina and Koroneiki volatiles presented inhibition zones against *P. savastanoi* according to diffusion method, but only stem volatiles of Arbequina inhibited visible growth of this bacterium at 125 µg/ml.

Similarly, Koroneiki volatiles, extracted from its flowers and stems, did not show inhibition zones against *A. tumefaciens* but presented nevertheless a visible inhibition growth at 125 and 225 µg/ml, respectively. The negative response of *A. tumefaciens*, when using the diffusion method, may be explained by the high resistance of these Gram-negative bacteria.

Additionally, the diffusion method can greatly vary according to the molecules [14], the organisms tested [15], and the inoculum size. Then, physical and chemical properties of the drugs as well as biological behavior of the bacteria could be put in competition, sometimes with a rather unpredictable outcome [16]. The volatiles were also tested qualitatively and quantitatively against several pathogenic fungi. All the tested volatiles exhibited moderate antifungal activities, with inhibition zones varying from 7.5 to 15 mm. These values were much smaller than those registered for the antifungal reference drug (45 to 57 mm).

However, all the Chemlali volatiles and Arbequina leaf volatiles exhibited a moderate antifungal activity against *P. italicum*, while the antifungal reference drug presented no activity against this species.

Differently, using the dilution method, almost all the olive volatiles exhibited interesting antifungal activities against the majority of fungi at low doses.

Growth of *F. solani* was totally inhibited by volatiles of Chemlali leaves, while growth of *A. niger* was totally inhibited by volatiles of Chemlali leaves and flowers. Additionally, *B. cinerea* was totally inhibited by volatiles extracted from Chemlali flowers. Consequently, Chemlali appeared to be the most active cultivar, totally inhibiting the growth of the three pathogenic fungi. *P. italicum* was totally inhibited by stem volatiles of the three olive cultivars, and by Arbequina flower volatiles. Thus *P. italicum* was the most sensitive species. The antifungal activity of olive volatiles, evaluated by diffusion method, was moderate.

However, this activity was more interesting when micro-dilution method was adopted, with low values of MIC and MFC. This proposed that the size of the inhibition zone does not reflect the real antibacterial efficiency of volatiles, since it is affected by the solubility of the oil, its diffusion in the agar, its evaporation, etc. This point was in agreement with Kim et al. [17] and Cimanga et al. [18] observations.

The essential oil activity is evidently related to the chemical composition of its compounds, their proportions and their interactions each other [19, 20].

Antifungal susceptibility is influenced by the type of medium, the inoculum size, the pH, the temperature and the time of incubation [21]. All tested samples exhibited an interesting antioxidant activity against DPPH radicals, reaching over 80% inhibition.

The most effective volatiles were those from stems of all the cultivars that inhibited 50% of radicals in the range 0.75-0.9 mg/ml.

Similarly, an important antioxidant activity was noted for all the volatiles when tested against the cation radicals ABTS•+, reaching 100% of radical inhibition for some of them. This activity depended on the tested organ, the cultivar and the contact time.

Leaf volatiles of Arbequina appeared to be the most active, scavenging the totality of radicals at only 1 mg/ml and in a very short time. Chemlali stems and Chemlali and Arbequina flowers were the most active against ABTS•+ when applied at low concentrations and short time of contact. Trolox equivalent antioxidant capacity measured after 30 min of contact presented elevated values, demonstrating the powerful antioxidant activity of these volatiles. Awika et al. [22] reported the advantage of ABTS•+ test over DPPH, as ABTS•+ test is operable over a wide range of pH, inexpensive and more rapid than the DPPH test. The absorbance of DPPH at 517 nm is depended on light, oxygen, pH and type of solvent [23]. Aruoma [24] mentioned that more than one method of antioxidant testing should be used to gain a perceivable indication of antioxidant efficacy of the tested substances. Chemlali, Arbequina and Koroneiki leaf, flower and stem volatiles exhibited interesting antioxidant and antimicrobial activities.

The chemical analyses evidenced the presence of several bioactive compounds. Indeed, all the volatiles contained hydrocarbons in important proportions (up to 49%). 1-Hexadecene, the main aliphatic hydrocarbon,
especially in Chemlali leaves (34.3%) is known for both antioxidant and antimicrobial activities [25-27]. Also terpenes were well represented among these olive cultivar volatiles, reaching a maximum of 73.9%.

Among apocarotenes, dihydroedulan IIA was particularly detected in Arbequina flowers (18.4%), while dihydroedulan IA was the major one in Koroneiki flowers (17.8%). Additionally, many other major bioactive apocarotens, exhibiting antioxidant and antimicrobial activities, were characterized, such as (E)-β-damascenone (Chemlali leaves, 16.8%) and (E)-geranylacetone (Koroneiki stems, 14.2%) [28-29] (Table 5).

Regarding oxygenated sesquiterpenes, the most representative ones were (E)-nerolidol (Koroneiki leaves, 13.1%) and ligulyl oxide (Arbequina stems, 11.0%) [30-31].

Some studies have proved that sometimes the whole volatile extracts have a more powerful biological activity compared to the major component [32-33]. These authors propose that the compounds present in the greatest proportions were responsible only for a part of the total activity; also, the other components present with smaller amount, contribute to the unregistered activity. As well, a synergistic effect between all components should be considered [34].

| Compounds          | Biological activity                          | References |
|--------------------|----------------------------------------------|------------|
| Hydrocarbons       |                                              |            |
| Hexadecene         | Antioxidant and antimicrobial activities      | [26]       |
| Nonane derivate    | Antioxidant and antimicrobial activities      | [35-36]    |
| Dodecane           | Antioxidant activity                          | [37]       |
| Trimethyldecane    | Antimicrobial activity                         | [38]       |
| Pentadecane        | Antimicrobial activity                         | [39]       |

| Oxygenated Monoterpenes |                                              |            |
|-------------------------|----------------------------------------------|------------|
| Linalool                | Antioxidant and antimicrobial activities      | [40-41]    |
| Terpineol              | Antioxidant and antimicrobial activities      | [42-43]    |

| Monoterpenes hydrocarbon |                                              |            |
|--------------------------|----------------------------------------------|------------|
| Carene                   | Antioxidant and antimicrobial activities      | [44]       |

| Oxygenated sesquiterpenes |                                              |            |
|---------------------------|----------------------------------------------|------------|
| Nerolidol                 | Antioxidant and antimicrobial activities      | [30; 45]   |
| Caryophyllene oxide       | Antioxidant and antimicrobial activities      | [46-47]    |
| Ligulyl oxide             | Antioxidant activity                          | [31]       |

| Apocarotenes              |                                              |            |
| β-Ionone                  | Antioxidant and antimicrobial activities      | [48-49]    |
| Dihydroedulan             | Antioxidant and antimicrobial activities      | [50-51]    |
| Beta-Damascenone          | Antioxidant and antimicrobial activities      | [28; 52]   |
| Geranylacetone            | Antioxidant and antimicrobial activities      | [53]       |
| Theaspirane               | Antioxidant activity                          | [54]       |
| Hexyl benzoate            | Antimicrobial activity                         | [55]       |
| Methyl 4-formylbenzoate   | Antimicrobial activity                         | [56]       |

| Aldehyde                  |                                              |            |
| Nonanal                   | Antifungal activity                           | [57]       |

| Fatty acid                |                                              |            |
| Nonanoic acid             | Antifungal activity                           | [58]       |
Conclusion

Chemlali, Arbequina and Koroneiki volatiles have shown an interesting antibacterial activity against dangerous pathogenic bacteria; in particular, the principal Tunisian cultivar Chemlali exhibited a powerful activity against *P. savastanoi* and *A. tumefaciens* at low concentration (125 µg/ml). Both Chemlali and Arbequina inhibited the visible growth of the majority of the tested fungi at 125 µg/ml through their leaf, stem and flower volatiles and could block completely their growth in many cases. Interestingly, all the tested olive volatiles have an excellent capacity to scavenge radicals. 50% of radicals were inhibited at 100 µg/ml by the Arbequina leaf volatiles which seemed to be the most active. Many bioactive compounds such as hydrocarbons, oxygenated monoterpenes and apocarotenes, have been identified in the olive cultivar volatiles. These components could contribute to the recorded activities, expected to be related to their stereochemistry, to the proportions in which they are recorded and to the interactions between them. Further research is required to elucidate the exact mode of action of these active principles. Thus, olive tree volatiles might be a prospective source of alternative antimicrobial and antioxidant agents interesting for a potential use in the biological control or the conservation of food products.

Conflict of Interest

The authors declare no conflict of interest.

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