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ORIGINAL PAPER

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CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITIES ASSESSMENT OF OLIVE FRUIT VOLATILES FROM DIFFERENT VARIETIES GROWN IN TUNISIA

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ABSTRACT

Volatile compounds, present in olives, are responsible for the olive fruit flavor and oil aroma, influencing the consumer's preference. These compounds have a biological activity to fight off pathogens. The aim of this work is to characterize volatiles in pulps and cores of Chemlali, Arbequina and Koroneiki olives, collected from Menzel Mhiri-Kairouan, and to test both the efficiency of these compounds against two bacteria and six phytopathogenic fungal species, by diffusion and dilution methods, and their antioxidants activities. The analyzis of volatiles were determined by GC-FID and GC-MS in three cultivars at the full ripening stage. Thirty five compounds were identified, such us an assortment of phenol, alcohol, hydrocarbon, aldehyde and terpenes derivatives. A high changes in volatiles was noted between cultivars and fruit organs. In fact, the major compounds in the pulps and the cores, of different cultivars, are (E)-2-decenal (46.9%), nonanal (19.6%), 1-hexadecene (16.3%), 7-methyl-1,3,5-cycloheptatriene 7-methyl-1,3,5-cycloheptatriene (15.47%), (E,E)-2,4-decadienal (14.5%) and 1-tetradecene (14.6%). Also, the cores volatiles illustrated more richness in aldehydes than the pulps for all cultivars. Volatile fractions exhibited a moderate to important antibacterial activities against bacteria. However, Arbequina cores volatiles and both Chemlali and Koroneiki pulps volatiles established a moderate to higher activities against tested fungi. The DPPH and $ABTS^{\bullet+}$ tests demonstrated that the highest antioxidant capacity of volatiles were assigned to Arbequina cores and Koroneiki pulps. The Principal Components Analysis showed a significant relationship between antioxidants and/or antimicrobial properties and the levels of the main volatile compounds (limonene, methyldecane, nonanal, E-2-decenal, camphor, geranic acid, tetradecene, hexadecane, tetradecane) in different fruit organs.

Key words: *Olea europaea* L., fruits, volatile compounds, antioxidant activity, antimicrobial property

INTRODUCTION

Various plant extracts, by their wealth in natural compounds, gotten of great interest. The valorization of these natural plant resources essentially involves the extraction of their essential oils. These are high value-added products used in the pharmaceutical, cosmetic and agri-food industries. Indeed, essential oils are a source of natural substances that has great potential for application, namely in agriculture, in the control of various microbes. These products have been selected for their efficiency against several diseases and their uses in the maintenance of alimentary compounds against the toxic effects of many oxidants [Bilel et al. 2015].

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Olea europaea is considered an excellent Mediterranean tree. Their fruits have an economic and alimentary importance; they provide various health benefits [Brahmi et al. 2013]. The fruits represent the most important organs of the olive tree and the source of virgin olive oil after mechanical extraction procedure. Their composition, especially volatile compounds, is largely affected by genetic factors, species, olive ripeness, climatic conditions [Angerosa et al. 2004]. Essential oils and aromas of fruits are derived from the secondary metabolism of the plant and can be stored in various structures such as epidermal cells, internal secretory cells. They are complex mixtures of volatile substances such as phenols, alcohols, aldehydes, terpenes and esters. Several researches [Angerosa et al. 2001, 2004] proved the increase of volatiles level during maturation process and reaches a maximum when the fruit skin turn to purple.

The use of secondary metabolites, antimicrobial and antioxidant properties, is one of the most interesting avenues to be explored in order to give better resistance to plants. Natural antioxidants, phenols, vitamins, enzymes and minerals are able to scavenging free radicals. They protect our organism cells from oxidant damage [Adorjan and Buchbauer 2010]. Olive fruit and their oil derivative was the source of natural antioxidant which may contribute to protect cellular components against the oxidant agent. In our days, natural compounds which have an antioxidants property are highly demanded by consumers for their potential to promote health, safety and reduce risk disease [Issaoui et al. 2009].

The volatile composition, antimicrobial and antioxidant activity of the olive fruits has been rarely established in the literature [Dabbou et al. 2011]. Our project is firstly interested to characterize qualitatively and quantitatively volatiles content in the pulps and the cores of fruits of three olive tree cultivars and their antioxidant activities and secondly, to test the behavior of essential oils of different organs against several bacteria and fungi, to study their possible uses in biological control.

MATERIAL AND METHODS

Experimental

Plant material. Samples were obtained from homogeneous olive fruits (*Olea europaea* L.) of three varieties, Chemlali, the main Tunisian oil cultivar and both the introduced cultivars, Koroneiki and Arbequina, cultivated in the same pedoclimatic conditions and growing in Menzel Mhiri – Kairouan (35'40"N, 10'06"E), in the Center of Tunisia. Olive fruits were harvested manually at full ripening stage during the 2012–2013 seasons in December.

Compounds extraction. After the manual separation of the core from the pulp, fresh fruit parts were carved in portions, weighted and placed forward to steam distillation for height hours. The distillate was removed by the hexane, dried with anhydrous Na_2SO_4, the solvent was separated with rotary evaporator and the volatile oils of each sample were stored at refrigerator previous to analysis.

Volatile compounds extraction

The extraction of volatile compounds of pulps and cores was determined by steam distillation. After the concentration of volatiles by rotary evaporator, the yield of each sample was determined by the ratio of the weight of volatile to the fresh weight of material.

Analysis of volatiles by gas chromatography

After injection of 0.1 µL of 1% of essential oil hexane solution, the volatiles were analyzed by apparatus Gas Chromatography (GC) equipped with flame ionization detectors (FID). Also, these compounds were determined by a Hewlett-Packard GC-MS system according to Saidana et al. [2008].

The identification of the volatile compounds was realized by the comparison of their mass spectra with those of a computer library. Each compound is confirmed by referring to Retention index (RI) data generated from n-alkanes series (C9-C28) [Adams 1995, Shibamoto 1987].

Antibacterial activity

Inoculums preparation. One day before testing, the suitable microbes were inoculated aseptically with Mueller–Hinton (M–H) broth. This test was to evaluate the growth state of bacteria and to verify their complete adaptation to the broth.

Antibacterial property test. The antibacterial activity of the volatile fractions was tested by disc diffusion [Marmonier 1987] and dilution methods [Burt 2004] against two selected species: *Agrobacterium tu-*

mefaciens (souche C58/ATCC 33970), *Pseudomonas sanastanoi* pv savastanoi (NCPPB 3335).

a) Diffusion method

The inoculums of pathogen bacteria were developed during one dayat 37°C in Mueller–Hinton (M-H) agar, this suspension restrained around 10^5 Colony Forming Unit (CFU)·mL^{-1} of bacteria.

The analyzes of antibacterial tests was realized by disc diffusion method described by Marmonier et al. [1987].

In sterile Mueller–Hinton agar, the 500 µL of the inoculums were spread above dishes and 0.025 mg/oil disc were placed in the filter paper discs. After incubation at 25°C for 24 h, the inhibition zone around the disc (six millimeter in diameter) was measured. In the same conditions, two controls were prepared; the first was the bacteria with solvent without extract and the second was ampicillin (5 mg·mL^{-1}) standard. The experiments were run in triplicate.

b) Dilusion method

The assessment of the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) were determined by the broth dilution method as described by Van der Berghe and Vlietinck [1991] and Burt [2004].

Antifungal activity

The study of the antifungal test was realized in six pathogenic species namely: *Fusarium solani, Aspergillus niger* (CBS 513.88/FGSC A1513), *Botrytis cinerea* (B05.10 et T4) *Trichoderma harzianum* T-22(KRL- AG2), *Phytophtora* sp. and *Neofusicocum* sp. These fungal species were cultivated in Potato Dextrose Agar (PDA).

The antifungal activity was tested by the disc and dilution methods according to Barry and Thornsberry [1991]. In this test, 10^7 CFU·mL^{-1} of fungal broth culture were used to evaluate the activity of the oils. Different concentration of the volatiles were impregnated with chloroform in filter paper and placed on the culture plates. Also, two wheels was tested, carbendazim (0.5 mg·mL^{-1}) as a positive control and chloroform as a negative control. After incubation for four days at 25°C, the inhibition zone of each disc was measured.

Antioxidant activity

The evaluation of antioxidant capacity of fruit pulps and stones extracts were tested by DPPH and ABTS radical assays.

DPPH radical-scavenging assay. The capacities of tested extracts, to scavenging free radicals, were examined by the method of Choi et al. [2002]. The ethanol solution of DPPH at 60 µM was prepared. The 0.5 mL of DPPH was added to 0.5 mL of the extracts at different concentrations (16; 8; 4; 2; 1; 0.5; 0.25 and 0.125 mg·mL^{-1}) and incubated in the dark for 30 min. The control contained 0.5 mL of DPPH solution and the same volume of ethanol. The absorbance was determined at 520 nm.

All the analyses were made in triplicate and the inhibition percentage of the DPPH radicals scavenging rate was determined as below:

$$\% \text{ DPPH radical scavenging activity (SC)} = = [1 - (A_{sample}/A_{control})] \times 100$$

Where $A_{control}$ is the absorbance of the control and A_{sample} is the absorbance of the tested sample after 30 min. The Trolox was used as a standard. Also, the IC50 of each sample was calculated, which represent the concentration of extract that could inhibit 50% of free radicals.

ABTS$^{\bullet+}$ radical assay. The ABTS radical, the second method to determine the antioxidant activity, was carried out according to Yu et al. [2002]. This method consists to oxidize the ammonium salt of 2,2-azinobis-3-ethylbenzthiazoline-6-sulphonic acid in ABTS radicals. Radical was prepared by oxidizing 100 µL of ABTS with 0.1 mg $K_2S_2O_8$. Volatile fractions were diluted in ethanol at different concentration 16; 8; 4; 2; 1; 0.5; 0.25 mg·mL^{-1}.

The analysis of the extracts was made by the mixture of 990 µl of ABTS solution and 10 µl of the tested extract. Furthermore, in the same conditions standard reaction was prepared (990 µl of ABTS and 10 µl of Trolox). All samples were vortexed for 10 s and the absorbance was determined at time intervals of 5, 10, 15, 20 and 30 min and examined with the control ABTS solution.

The antioxidant activities of extracts were expressed in TEAC (Trolox Equivalent Antioxidant

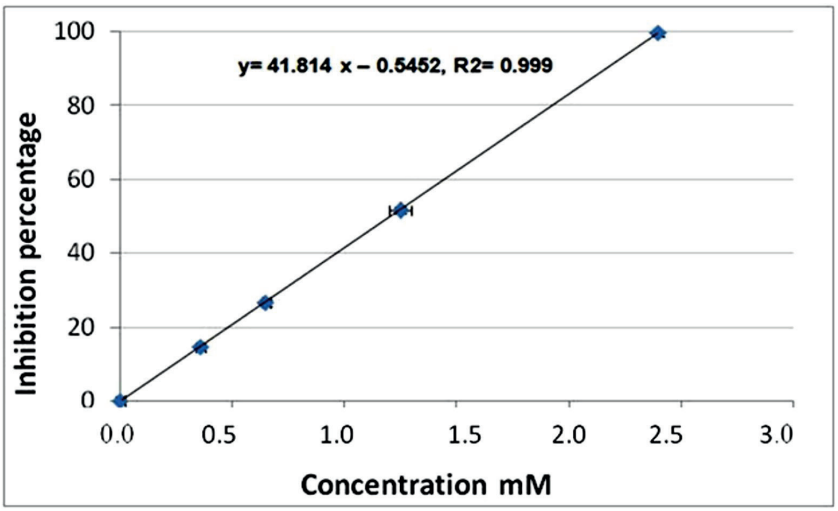


Fig. 1. Reference curve of Trolox

Capacity) and compared to Trolox equivalent con tent in 1 mM extract. All experiments were carried out in triplicate.

The values of TEAC were calculated according to the equation of Trolox curve (Fig. 1).

Statistical analysis

The determinations on all bioassays were carried out in triplicates and the values were mean ± standard deviations. The statistical analyzes were carried out using SPSS 20.0 and Microsoft Excel programs. Variance Analysis was accomplished by ANOVA procedures. Significant differences between means were determined by the Student's test, with significance at $p < 0.05$.

Principal Component Analysis (PCA). The main object of PCA was to reduce the number of variables in pulps and cores of olives from cvs Arbequina, Koroneiki and Chemlali (variables corresponding to the amount of most abundant volatile compounds identified in olive organs and their antimicrobial and antioxidant levels), to a reducer number of new derived components. These parameters were represented by plotting them in a multidimensional space, summarizing sufficiently the original information. PCA and person's correlation were performed by using SPSS software, version 20.

RESULTS

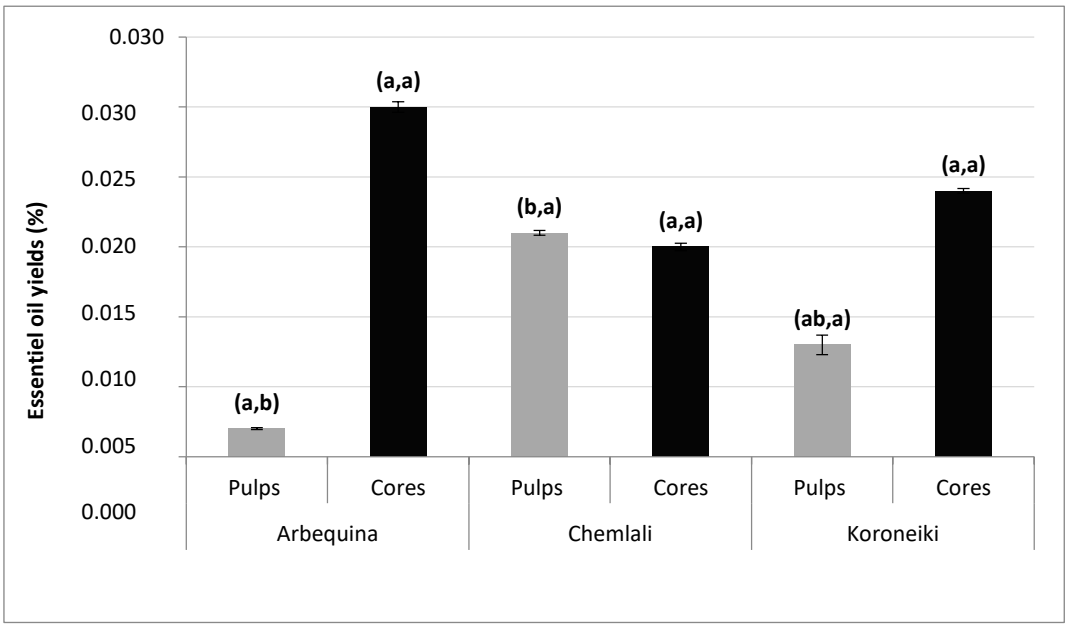
Yield of volatile fractions

The yield of the essential oil fractions obtained from Chemlali, Arbequina and Koroneiki cultivars during the extraction process are illustrated in Figure 2 and 3.

The yields of essential oil were widely variables depending on the variety and the plant material used. Chemlali represents the most interesting pulps yield (0.016%), compared to other studied varieties (Fig. 3). Olive pulps and cores yield don't have the same amount of volatiles. Furthermore, when comparing yields from three varieties, we find that the most important value was represented by Arbequina cores with 0.025%. While, Koroneiki and Chemlali showed a 0.019 and 0.015% respectively in essential oil yields.

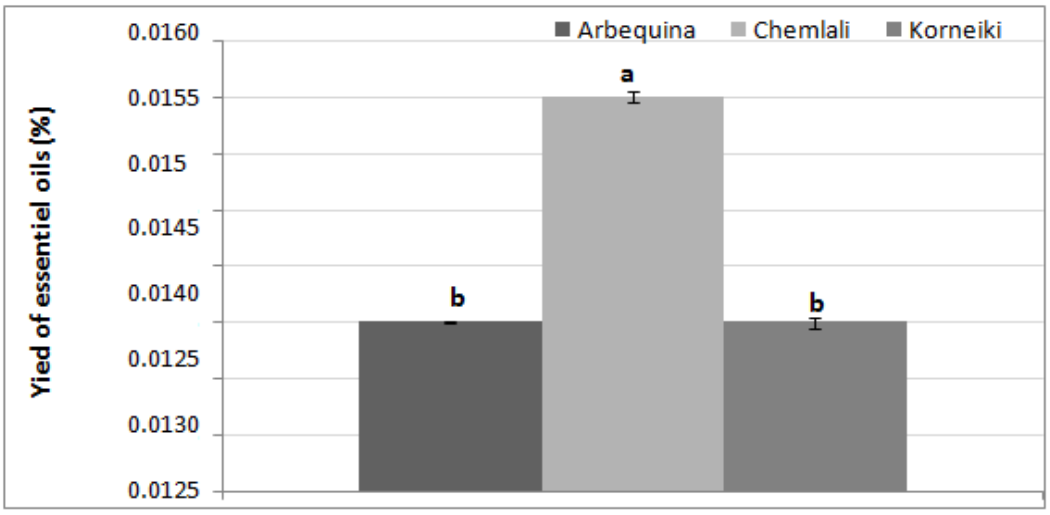
The results showed that for different parts of three varieties of olive fruit, essential oil yield was always higher in the cores than in the pulps except for Chemlali, no significant differences between the cores and the pulps. Therefore for Arbequina, the essential oil yield of cores (0.025%) was 12 times higher than that of the pulps (0.002%).

The olives from Chemlali variety appeared richer in essential oils with a yield up to 0.015%. Both varieties Arbequina and Koroneiki have the same levels of essential oils (Fig. 3).



Same letter: no significant difference, change of letter: significant difference at 5% levels. The first letter corresponds to the varietal comparison in mean contents of essential oils of the same organ, the second letter corresponds to the comparison between the average contents of the organs within the same variety

Fig. 2. Quantitative comparison in essential oils of pulps and cores of olive tree cultivars, Chemlali, Koroneiki and Arbequina



Means in the same letter are not significantly; means in different letter are significantly different ($p < 0.05$)

Fig. 3. Yield of essential oils of fruits from Chemlali, Koroneiki and Arbequina

Volatiles composition

The volatile composition of *Olea europaea* L. Chemlali, Arbequina and Koroneiki cores and pulpsare illustrated in Table 2. In fact, thirtyfive compounds were identified by gas chromatography-mass spectrometry analysis, as an assortment of alcohols, hydrocarbons, phenols, aldehydes, and terpene derivatives. Nonanal and (E)-2-decenal were only identified in volatiles of all organs of three cultivars. Moreover, (E)-2-decenal was the prominent component in both the pulps (15.4%) and the cores (46.9%) of Arbequina volatiles. The most abundant volatiles from the cores of olive cultivars were (E)-2-decenal (46.9%), nonanal (19.6%), 1-hexadecene (16.3%), 7-Methyl-1,3,5-Cycloheptatriene (15.47%), *(E,E)*-2,4-decadienal (14.5%) and 1-tetradecene (14.6%).

The (Z)-2-heptenal, limonene, 1-tetradecene and 1-hexadecene were identified in all studied organs of Arbequina and Koroneiki fruits but these compounds were absent in Chemlali ones. While, only in both Chemlali cores and pulpsheptadecane, *n*-undecane, 2,4,6,8-Tetramethylundeceneand decanewere to be found.In fact, Chemlali cores showed a richness in *(E,E)*-2,4-decadienal (10.7%), 7-methyl-1,3,5-cycloheptatriene (15.47%), 2–7 dimethyloctane (8.14%), 2-methyltetradecane (8.14%), 1-octanol (6.45%) and 2-methyl-Eicosane (2.56%). Also, 2-methyldodecane, 1-(pentyloxy)-hexane, and 2,3,4-trimethylhexane were present only in Chemlali pulps. Even as, 2–7 dimethyloctane (8.14%), 7-methyl-1,3,5-cycloheptatriene (15.47%) and 2-Methyltetradecane (8.14%) appeared to be unique to Chemlali cores.

We noted that volatiles from Koroneiki pulps showed a richnessin camphor (11.6%), 2,6,11-trimethyldodecane (9.1%), n-hexadecane (9.9%) and geranic acid (7.9%). But in the cores of Koroneiki, nonanal (19.6%), (E,Z)-2,4-decadienal (11.4%), (E,E)-2,4-decadienal (14.5%), 1-tetradecene (14.6%), 1-hexadecene (16.3%), (E)-2-decenal (7.9%) and camphor (8.9%) were the major components. In Arbequina, the cores explained a higher level of (E)-2-decenal (46.9%), six times more important than in koroneiki and ten times in Chemlali ones.

Antibacterial activity

The activities against bacteria,of pulps and cores volatiles of Chemlali, Korneiki and Arbequina, tested by disc diffusion and dilution methods, were illustrated in tables 2 and 3. The results were evaluated by the existence or nonappearance of inhibition zones, zone diameters, MIC and MBC values.

Diffusion method. Volatile fractions endowed with a moderate to important antibacterial activities against all bacteria (Tab. 2). The Chemlali pulps and cores volatiles showed a moderate antibacterial activity against *Agrobacterium tumefaciens* and *P. savastanoi* pv savastanoi. While, the Arbequina pulps volatiles showed a higher activity (14.5 mm inhibition area) against *Pseudominas savastanoi* pv savastanoi. However, Koroneiki pulps and cores volatiles exhibited a high and moderate activity against bacteria *P. savastanoi* pv savastanoi and *Agrobacterium tumefaciens* respectively.

Dilution method. The results illustrated in Table 3 showed that the oils from all tested cores exhibited an interesting antibacterial activity against *Pseudomonas savastanoi* pv. Moreover, all pulp volatiles, except Koroneiki pulps, endowed with a moderate activity against this bacterium for different concentrations. While, *Agrobacterium tumefaciens* was resistant against all volatiles of three cultivars.

Biological assays showed that *Pseudomonas savasanoi* pv savastanoi prevented visible growth of tested bacteria at the concentration 0.25 $mg \cdot mL^{-1}$ for cores and 1 $mg \cdot mL^{-1}$ for Chemlali and Arbequina pulps. While, this bacteria resisted to Koroneiki pulp volatiles. Arbequina and Chemlali volatiles appear to be more active against this bacterium. On the other hand, *Agrobacterium tumefaciens* appears resistant to all tested volatile oils, where no inhibitor activity appears.

Antifungal activity

To determine their powers fungicides, volatile fractions extracted from pulps and cores of Koroneiki, Arbequina and Chemlali fruits were tested at different concentrations/disk against *Trichoderma harzianum*, *Aspergillus niger*, *Phytophthora* sp., *Neofusicocum* sp., *Botrytis cinerea* and *Fusarium solani*. The results are expressed in Table 4 and 5. The volatile fractions of Chemlali pulps, Arbequina cores and Koroneiki pulps showed an antifungal activity against *Neofusicom* sp., *Phytophthora* sp. and *Trichoderma harzianum*. The inhibitions zones varied from 6.5 mm to 17.75 mm.

Table 1. Volatile compounds (%) evaluated by GC-FID and GC-MS in the cores and the pulps of Chemlali, Koroneiki and Arbequina grown in the studied area. Percentages obtained by FID peak area normalization

Volatile compounds		Arbequina		Koroneiki		Chemlali	
Organs	[b]RI	cores	pulps	cores	pulps	cores	pulps
2,3,4-Trimethylhexane	850						2.63 ±0.03
7-Methyl-1,3,5-cycloheptatriene	890					15.47 ±0.07	
(Z)-2-heptenal	927	7.9 ±0.43	4.0 ±0.1	1.3 ±0.1	1.2 ±0.1		
2,7- dimethyloctane	929					8.14 ±0.08	
Decane	1000					2.03 ±0.15	1.21 ±0.015
Limonene	1032	1.0 ±0.1	1.6 ±0.21	1.2 ±0.21	1.1 ±0.17		
2-methyldecane	1062		4.5 ±0.015		5.8 ±0.09		
1-Octanol	1069		0.4 ±0.05			6.45 ±0.07	
n-undecane	1072		1.4 ±0.1		2.0 ±0.05	2.41 ±0.05	9.74 ±0.06
3-Ethyl-o-xylene	1094					1.22 ±0.03	
Linalool	1100				0.6 ±0.05		0.9 ±0.043
Nonanal	1103	7.7 ±0.12	3.1 ±0.06	19.6 ±0.1	5.1 ±0.05	3.5 ±0.1	1.7 ±0.07
Camphor	1145		5.3 ±0.07	8.9 ±0.46	11.6 ±0.4		
1-dodecene	1193		3.2 ±0.12		3.5 ±0.1		
n-dodecane	1200		0.6 ±0.01		0.6 ±0.012		
Dodecane, 1,1-difluoro	1202					1.38 ±0.01	
Decanal	1205		1.3 ±0.05		1.0 ±0.01		
(E)-2-decenal	1263	46.9 ±0.43	15.4 ±0.16	7.9 ±0.43	4.9 ±0.1	4.5 ±0.12	1.72 ±0.02
2-methyldodecane	1265						11.06 ±0.03
2,6,11-trimethyldodecane	1277		5.7 ±0.1		9.1 ±0.05		
(E,Z) -2,4-decadienal	1295	6.2 ±0.1	1.8 ±0.1	11.4 ±0.2		7.6 ±0.02	
(E,E)-2,4-decadienal	1317	8.1 ±0.03	6.2 ±0.05	14.5 ±0.1		10.7 ±0.41	
2,4,6,8-Tetramethylundecene	1321					0.74 ±0.03	5.6 ±0.2
Geranic acid	1357				7.9 ±0.05		
1-tetradecene	1392	9.0 ±0.02	5.1 ±0.05	14.6 ±0.15	11.0 ±0.2		
n-tetradecane	1400		3.5 ±0.43		5.1 ±0.05		
2-Methyltetradecane	1464.9					8.14 ±0.03	
(E, E)-α-farnesene	1507		19.8 ±0.1		4.5 ±0.05		7.9 ±0.05
1-hexadecene	1593	10.3 ±0.1	8.0 ±0.05	16.3 ±0.5	11.3 ±0.1		
n-hexadecane	1600		6.9 ±0.05		9.9 ±0.05		
2-Octyldodecanol	–						0.62 ±0.01
Heptadecane	1700					8.87 ±0.01	10.42 ±0.02
Hexane, 1-(pentyloxy)	–						2.51 ±0.01
Eicosane, 2-methyl-	2064					2.56 ±0.07	
Total		97.1 ±0.9	97. 8 ±1.9	95.7 ±1.7	96. 2 ±1.57	83.71 ±1.06	56.01 ±1.01

[a] Percentages obtained by FID peak area normalization (HP-5 column). [b] RI (DB-5 column). The values represent the mean of three replicates ± SD

Table 2. Antibacterial activity of volatile oils of three varieties of *Olea europaea*: Chemlali, Arbequina, Koroneiki Pulpsand cores (diffusion method) against pathogenic bacteria (mg·mL^{-1})

Bacterial species		Chemlali		Arbequina		Koroneiki		Negative control	Positive control
		pulps	cores	pulps	cores	pulps	cores		
	Concentration (mg·mL^{-1})	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0
Pseudomonas savasanoi pv savastanoi *(NCPPB 3335)*	Presence or absence of inhibition zones (+/–)	(+)	(+)	(+)	(–)	(+)	(–)	(–)	(+)
	Zone diameters (Φ mm)	8	7	14.5		8.5			20
Agrobacterium tumefaciens (C58/ATCC 33970)	Presence or absence of inhibition zones (+/–)	(+)	(+)	(–)	(–)	(–)	(+)	(–)	(+)
	Zone diameters (Φ mm)	8	8				12		23.5

Two references were used for bacteria tests; ampicillin and chloroform were used to positive and negative control respectively. The values represent the mean of three experiments

Table 3. Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of the volatiles of pulps and cores of three varieties of *Olea europaea*: Chemlali, Arbequina and Koroneiki against pathogenic bacteria (mg·mL^{-1}) dilution method

Bacterial species	Inhibitrice activity	Concentrations (mg·mL^{-1})	Chemlali				Arbequina				Koroneiki			
			pulps		cores		pulps		cores		pulps		cores	
			MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Pseudomonas savasanoi pv savastanoi (*NCPPB3335*)	+	1	+	>1	+	>1	+	>1	+	>1	–	–	+	>1
		0.5	–	>1	+	>1	+	>1	+	>1	–	–	+	>1
		0.25	–	>1	+	> 1	–	>1	+	>1	–	–	+	>1
		0.125	–	>1	–	>1	–	>1	–	>1	–	–	–	>1
Agrobacterium tumefaciens (*C58/ATCC 33970*)	–	1	–	–	–	–	–	–	–	–	–	–	–	–
		0.5	–	–	–	–	–	–	–	–	–	–	–	–
		0.25	–	–	–	–	–	–	–	–	–	–	–	–
		0.125	–	–	–	–	–	–	–	–	–	–	–	–

Table 4. Antifungal activities of cores and pulps volatile oils of Chemlali, Arbequina and Koroneiki – disc diffusion method

Varieties		Diameter of inhibition zone							
		Chemlali		Arbequina		Koroneiki			
Organs		pulps	cores	pulps	cores	pulps	cores		
Concentration of essentiel oils		0.025	0.025	0.025	0.025	0.025		Negative control	Positive control
Neofusicocum sp.	+/-	(+)	(-)	(+)	(+)	(+)	(-)	(-)	(+)
	Φ (mm)	16.75		11	17.75	16.25			
Phytophthora sp.	+/-	(+)	(-)	(-)	(+)	(+)	(-)	(-)	(+)
		8.5			7.5	7.5			
Trichoderma harzinum	+/-	(+)	(-)	(-)	(+)	(+)	(+)	(-)	(+)
	Φ (mm)	10.25			9.75	10.25	8.5		50
Aspergillus niger	+/-	(-)	(-)	(-)	(+)	(-)	(-)	(-)	(+)
	Φ (mm)				7.5				32.5
Botrytis cinerea	+/-	(+)	(-)	(-)	(+)	(-)	(-)	(-)	(+)
	Φ (mm)	6.75			6.5				8.5
Fusarium solani	+/-	(-)	(+)	(+)	(+)	(-)	(-)	(-)	(+)
	Φ (mm)		2	2.3	2.8				4

Carbendazim (anti-fungal agent) and chloroform were used as references for positive and negative control, respectively. The values represent the mean of three measurements

+/– presence or absence of inhibition zone, Φ – diameter of inhibition zone (mm), (+) presence of a fungicidal activity, (–) absence of a fungicidal activity

Table 5. Minimal inhibitory concentration (MIC) and minimal fungal concentration (MBC) of the volatile oils of three varieties of *Olea europaea* against some pathogenic fungal ($mg \cdot mL^{-1}$) – dilution method

			Diameterofinhibition zone (mm)											
			Chemlali				**Arbequina**				**Koroneiki**			
			Pulps		**cores**		**Pulps**		**cores**		**Pulps**		**cores**	
Name of microorganism	**Inhibition activity**	**Concentration**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
Aspergillus niger	+	1	-	-	+	>1	-	-	-	+	-	-	-	-
		0.5	-	-	-	>1	-	-	-	+	-	-	-	-
		0.25	-	-	-	>1	-	-	-	+	-	-	-	-
		0.125	-	-	-	>1	-	-	-	+	-	-	-	-
Botrytis cinerea	+	1	+	>1	+	>1	+	>1	+	>1	+	>1	-	-
		0.5	-	>1	+	>1	+	>1	+	>1	-	>1	-	-
		0.25	-	>1	+	>1	+	>1	+	>1	-	>1	-	-
		0.125	-	>1	-	>1	-	>1	-	>1	-	>1	-	-
Trichoderma harzianum	+	1	-	-	-	-	+	>1	+	>1	+	>1	+	>1
		0.5	-	-	-	-	+	>1	+	>1	-	>1	-	>1
		0.25	-	-	-	-	-	>1	+	>1	-	>1	-	>1
		0.125	-	-	-	-	-	>1	-	>1	-	>1	-	>1
Fusarium solani	+	1	-	-	+	>1	+	>1	+	>1	-	-	-	-
		0.5	-	-	-	>1	-	>1	+	>1	-	-	-	-
		0.25	-	-	-	>1	-	>1	-	>1	-	-	-	-

The growth of all fungal strains tested appears to be inhibited by the essential oils of Arbequina cores (Tabs. 4 and 5). In fact, the minimum bactericidal concentration (MBC) of this fraction is equal to 0.125 mg·mL^{-1} against *Aspergillus niger*. Similarly, this strain appears sensitive also against Chemlali cores; the minimum inhibitory concentration (MIC) was 1 mg·mL^{-1} but resistant to volatile fractions of other tested varieties (Tab. 5). The Arbequina cores volatiles seems to be gifted powerful antifungal for *Neofusicomsp* fungus, presenting an inhibition area reaching 17.75 mm. Whereas, moderate antifungal activities were detected against other species (between 2.8 and 9.75 mm).

The *Botrytis cinerea* strain appeared sensitive to essential oils of Chemlali and Koroneiki pulps with MIC of 1 mg·mL^{-1} and Chemlali and Arbequina cores with MIC of 0.25 mg·mL^{-1} respectively. However, *Trichoderma harzianum* was resistant to essential oils of Chemlali. For against fractions with other varieties, *Trichoderma* seemed to be sensitive with MIC of 0.25 mg·mL^{-1}, 0.5 mg·mL^{-1} for the cores and the pulps of Arbequina, respectively. As well, this fungal was sensitive against Koroneiki pulps and cores volatiles with a MIC of 1 mg·mL^{-1}. Also, the volatile oils of Chemlali cores have moderate activity against *Fusarium solani*.

Antioxidant activity

The evaluation of the antioxidant capacity of different organs of Chemlali, Arbequina and Koroneiki volatiles, two assays were used: DPPH and ABTS radical assays.

DPPH radical-scavenging assay. The antioxidant effects, of volatile compounds, in DPPH test, reflect-the capacity of an element tocede hydrogen and neutralize free radicals. The DPPH-radical-scavenging activity of volatile oils of pulps and cores of Chemlali, Arbequina and Koroneiki is illustrated in Table 6. Volatile fractions of different studied varieties, exceeding 1mg·mL^{-1}, were active against DPPH radicals. Indeed, at high concentrations, 16 and 8 mg·mL^{-1}, the percentage of scavenging activity on DPPH radicals was over 60% except Chemlali cores fraction. The inhibition level of free radicals was about of 78.46; 74.83; 74.26; 71.87% corresponding to fractions extracted fromArbequina and Koroneiki cores, Chemlali and Koroneiki pulps respectively. At low concentrations, 0.25 mg·mL^{-1}, Arbequina cores and Koroneiki pulps fractions showed a higher performance in scavenging radicals comparatively to other volatile fractions, 29.93% and 31.66% of antioxidant activity were unregistered respectively.

Table 6. DPPH radical scavenging activities (%) of Chemlali, Arbequina and Koroneiki volatile fractions and Trolox, the standard (mean ± SD)

		Concentrations of volatile fractions (mg.mL^{-1})							
Varieties	**Organs**	**16**	**8**	**4**	**2**	**1**	**0,5**	**0,25**	**0,125**
Chemlali	**Pulps**	74.26 ±0.22c	66.63 ±3.07c	42.82 ±2.50b	26.88 ±0.22a	20.05 ±0.80a	19.59 ±0.66ab	9.45 ±0.4a	5.58 ±0.18a
	Cores	52.72 ±0. 97a	38.44 ±1.92a	28.91 ±1.05a	23.13 ±0.13a	18.37 ±0.13a	14.85 ±0.82a	7.26 ± 0.65a	9.07 ±0.2ab
Arbequina	**Pulps**	65.75 ±3.93b	60.02 ±4.41b	43.08 ±0.59b	29.95 ±0.77a	21.48 ±0.12a	19.57 ±0.13ab	15.04 ± 0.85a	8.59 ±0.03ab
	Cores	78.46 ±1.84d	72.56 ±2.40d	59.41 ±4.08c	54.31 ±0.24b	31.97 ±0.6ab	37. 87 ±0.74b	29.900 ±0.27b	18.14 ±0.80c
Koroneiki	**Pulps**	68.79 ±0.16b	71.87 ±0.56cd	61.96 ±0.91c	53.87 ±0.56b	40.5 ±0.2b	36.10 ±0.40b	31.66 ±0.25b	23.01 ±0.27c
	Cores	74.83 ±0.55c	68.46 ±3.13cd	64.09 ±0.44c	47.65 ±0.44b	27.40 ±0.27ab	18.23 ±0.11ab	13.98 ±0.56a	11.07 ±0.10ab
Trolox		100.00 ±0.00e	100.00 ±0.00e	94.45 ±0.05d	94.98 ±0.10c	95.32 ±0.22c	95.39 ±0.11c	94.91 ±0.50c	94.70 ±0.11d

Different letters in the same colon concerning samples at the same concentration, represent significant differences between three treatments according to Duncan's multiple range test at $P < 0.05$

Moreover, the lower IC_{50} value indicates higher antioxidant activity. The pulps of Koroneiki (1.3 mg·mL^{-1}) and the cores of Arbequina showed the highest scavenging activity on DPPH and ABTS radicals. While, the highest IC_{50} value is illustrated in volatile oil from Chemlali Cores. The results showed that pulps of Koroneiki and the cores of Arbequina seemed to be the most active organ compared to other studied organs and varieties.

ABTS$^{\bullet+}$ scavenging assay. The free radical scavenging properties of the fresh pulps and cores volatiles of Chemlali, Arbequina and Koroneiki are presented in Figure 4. All tested volatiles, from the pulps and cores of three species, exhibited a low to high scavenging efficiency toward ABTS radicals. The volatile fractions of pulps and cores of Chemlali, at 8 and 16 mg·mL^{-1}, were the most active against ABTS$^{\bullet+}$ radicals when compared to Arbequina and Koroneiki. Furthermore, the antioxidant activities of volatile compounds of Chemlali pulps (69% inhibition for 16 mg·mL^{-1} at 30 mn incubation times) appear more active than its cores. The figure 3 showed that the highest radicalscavenging activity was determined by Arbequina pulp volatiles. While, at low concentrations of volatile fractions, lower than 0.25 mg·mL^{-1}, and for all incubation times Koroneiki pulps exhibited the uppermost antioxidant capacity for scavenging radicals.

The TEAC value indicates the equivalent antioxidant capacity of Trolox, expressed the concentration of Trolox, which had the same antioxidant capacity at 1 mM concentration of a tested sample. Concerning the extracts, a higher TEAC would imply greater antioxidant activity of the sample. After 30 min of the reaction, TEAC of each volatile was determined (Tab. 7). The result showed that Chemlali pulp volatiles had a higher TEAC about of 1.67 mM at the concentration of 16 mg·mL^{-1}. The TEAC values of the other volatile oils at this concentration ranged from 1.27 to 1.50 mM.

Principal component analysis

To recapitulate all collected data, a principal component analysis (PCA) were performed with the relative proportion of the volatile compounds content, in olive fruits organs of three cultivars, with their antioxidant and antimicrobial properties. The cumulative levels of variance described by the two first principal components (F1 with 35.18% and F2 with 25.18%) explained 60.36% (Fig. 5). The nonanal and E-2-decenal, the two most important volatiles, were related with antifungal activity, against *Aspergellus*, *Botritys* and *Fusarium* levels, which apparent clearly in the PCA analysis. These two volatiles were mainly correlated with antifungal properties of samples from Koroneiki and Arbequina cores. The E-2-decenal was highly and significantly correlated with antifungal properties against *Aspergellus*, *Botritys* and *Fusarium*, which was 0.97**, 0.60** and 0.70** respectively essentially from Koroneiki cores and Arbequina pulps and cores (Tab. 7). Also, according to PCA analysis (E,E)-2,4-decadienal and (E,Z)-2,4-decadienal were significantly correlated with antibacterial activity against *Agrobacterium* (0.59** and 0.51** respectively). As regards, the (E,E)-α-farnesene, n-tetradecane, geranic acid, 2,6,11-trimethyldodecane, 1-dodecene, a positive and significant relationship were also established between its relative abundance in Koroneiki pulps and the antibacterial and antifungal activities levels against *Pseudonomas*, *Phytophtora, Neofusicoccum*, *Trichoderma* species. Also, PCA analysis showed that these compounds were negatively and significantly correlated with IC50 (of both DPPH and ABTS radicals) which determined a strong capacity to scavenging free radicals. These antioxidants activities were related especially to pulps and cores from cvs. Arbequina and Koroneiki. The PCA analysis noted a difference in Chemlali volatiles to other olive cultivars which are represented by the abundance of hydrocanbons in the extreme opposite area to antimicrobial activities.

DISCUSSION

In the present study, a difference in volatile fractions was observed between varieties and fruit organs. The average yields of olive essential oils varied between 0.002 and 0.025%. This result was in accordance with those of Brahmi [2012] that the yield of volatile fractions of Chemlali, obtained by steam distillation, was of 0.033%. In any study, it seems that variation in volatile yields can be attributed to some factors like varieties, organs and culture condition [Brahmi et al. 2013].

The qualitative and quantitative analysis of volatiles, by GC/MS, showed that the genotype, the fruit

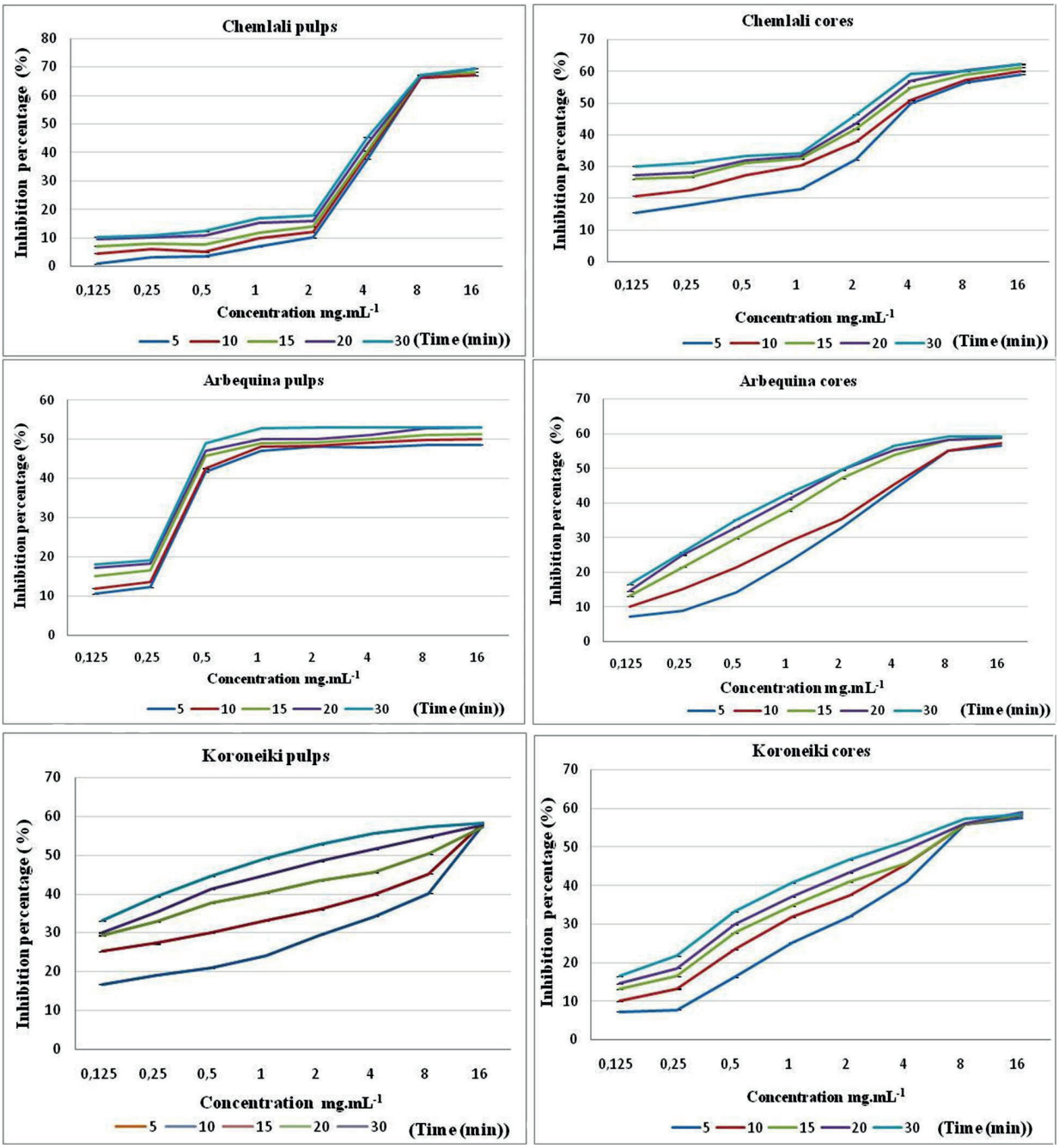


Fig. 4. $ABTS^{\bullet+}$ cation radical scavenging capacity of volatile compounds extract of cores and pulps of three cultivars as a function of time and concentrations

organs and the growing conditions have a large effect on the oil composition [Saidana et al. 2008, Piccaglia et al. 1991]. Comparing the three cultivars, Chemlali (local variety), Koroneiki and Arbequina (Introduced varieties) a significant difference between pulps and cores volatiles was determined.

In the present study, the pulps and cores volatiles of three cultivars showed the presence of saturated and unsaturated compounds such as, aldehydes, hydrocarbons, alcohols, phenols and terpenes. The fruit volatiles of three cultivars showed richness in aldehydes such us nonanal, (E)-2-decenal and 2, 4-decadienal; while, Koroneiki and Arbequina were more representative by these compounds. As shown in an earlier study [Ben Temime et al. 2006, Ben Mansour-Gueddes et al. 2018] the main constituents identified in Chetouioil volatiles were aldehydes, hydrocarbons, alcohols, terpenes and esters. Also, the study of Montedoro et al. [1978] showed that aldehydes correspond to the main aroma components in olive leaves, fruit and oil: their content in olives vary among 50% and 75%. The production of these components was attributed by the biogenic ways of the olive, such as the lipoxigenase (LOX) pathways, the metabolism of fatty acids or amino acids [Morales and Przybylski 2013, Brahmi et al. 2011]. Also, Malheiro et al. [2015] suggested the polyunsaturated fatty acids are oxidized by LOX and cleaved by the presence and the activity of many enzymes; this proves, therefore, the performance of lipoxygenase enzymes on unsaturated acids. Thus, depending on the polyunsaturated fatty acid intervened, different volatiles are formed [Malheiro et al. 2015]. For this reason, wide range of volatile compounds, constitute the profiles of three cultivars, such as nonanal, (E)-2-decenal and (E,E)-deca-2,4-dienal were the oxidation products of oleic and linoleic acids [Sansone-Land et al. 2014]. The results showed that the oxidation of these fatty acids is carried out essentially at the level of the fruit stones. Also, the existence of high level of nonanal, the oxidation product of oleic acid, in Arbequina and Koroneiki cores could be explained by their higher antimicrobial activity [Bassole et al. 2003]. Also, Cao et al. [2014] showed the level of nonanal in camellia oil (oleic acid mainly) increased and correlated significantly with the total oxidation values.

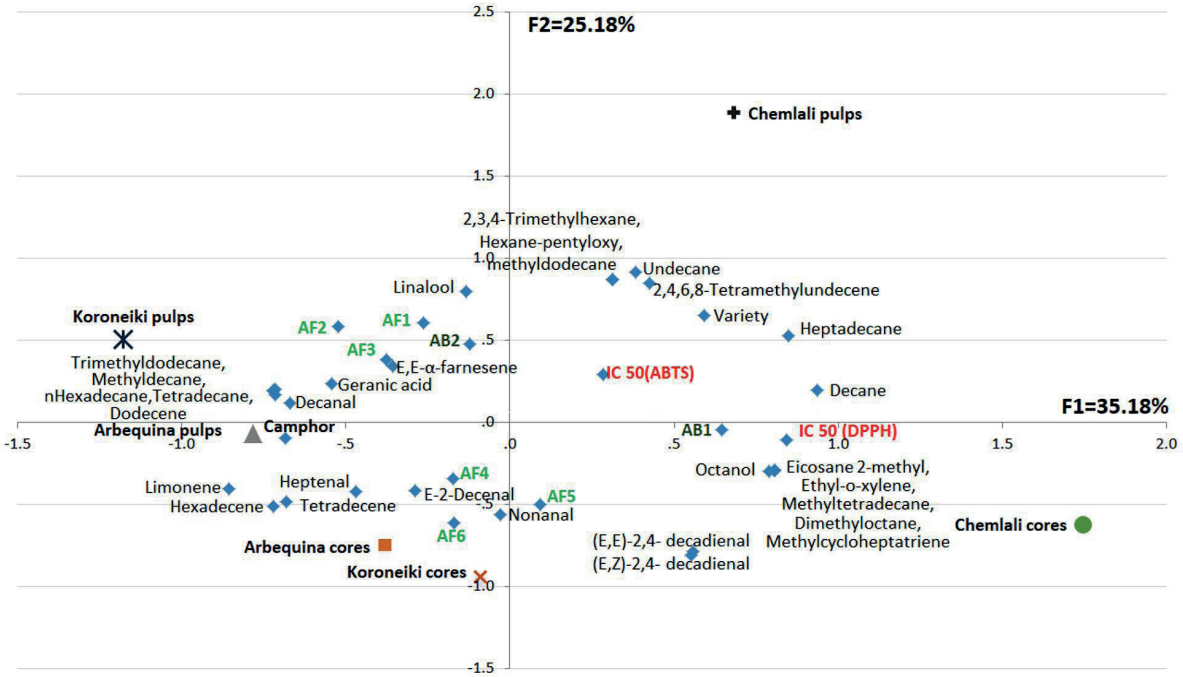


Fig. 5. Principal Component Analysis (PCA) of the main groups of volatile compounds of fruits from Arbequina, Koroneiki and Chemlali cultivars and their antimicrobial and antioxidant activities. Bi-plot of the main groups of compounds in the essential oils from the cores and the pulps of three cultivars. Factors 1 and 2 explained 60.36% of the data variation. AB1: Anti-*Agrobacterium tumefaciens*, AB2: Anti-*Pseudonomas savasanoi* pv, AF1: Anti-*Phytophtora* sp., AF2: Anti-*Neofusicoccum* sp.; AF3: Anti-*Trichoderma harzianum*; AF4: Anti-*Aspergellus niger*; AF5: Anti-*Fusarium solani*; AF6: Anti-*Botritys cinerea*. IC50 (ABTS): Inibitrice concentration of 50% of ABTS radicals; IC50 (DPPH) inibitrice concentration of 50% of DPPH radicals

Table 7. The Inhibition concentration (IC50) of Chemlali, Koroneikiand Arbequina volatile fractions

Varieties	Organs	DPPH $IC_{50(mg.mL^{-1})}$	$ABTS^{\bullet+}$ $IC_{50(mg.mL^{-1})}$	TEAC(mM)
Chemlali	Pulps	3.8±0.03	4.67±0.02	1.67
	Cores	11.0±0.07	2.58±0.02	1.50
Arbequina	Pulps	3.9±0.04	2.07±0.05	1.40
	Cores	1.4±0.02	2.01±0.03	1.41
Koroneiki	Pulps	1.3±0.01	1.30±0.03	1.27
	Cores	1.7±0.05	3.62±0.02	1.42

[b] Values are means of three replicate determinations ± standard deviation
TEAC – Trolox equivalent antioxidant capacity

While, Chemlali showed the lowest level of nonanal. This result could indicate that the unsaturated fatty acids were less oxidable in Chemlali than in Arbequina and Koroneiki samples. Moreover, we noted that the high level of (E,E)-2,4-decadienal, relative to linoleate oxidation products, in Arbequina cores and pulps and the cores of Koroneiki and Chemlali indicated a high oxidation of the linoleic acid.

The antibacterial activity of the volatile oils against *Pseudomonas savasanoi* pv savastanoi and *Agrobacterium tumefaciens* was assessed. Only volatiles of Koroneiki cores and Chemlali pulps and cores volatile fractions exhibited antimicrobial activity against *Agrobacterium tumefaciens*. Whereas, pulp and stone volatiles revealed a moderate to important antimicrobial property against *P. savasanoi* except Koroneiki cores. According to Chang et al. [2001] this activity could be credited to the existence of a high level of aldehydes such as nonanal and (E)-2-decenal, of terpenoids such as (E,E)-α-farnesene [Dabbou et al. 2012], and camphor [Ben Mansour et al. 2015]. The latest compounds concentrated especially in the pulps of Koroneiki and Arbequina volatiles which were known to show a significant antibacterial activity [Fahmidabinti et al. 2016]. However, the entire volatile fraction is, in most cases, more active than its main components. This recommended that the involvement of minor compounds must be examined as well as the synergistic effect among all constituent [Gill et al. 2002].

Furthermore, according to the both analysis of disc diffusion and dilution tests, for the antibacterial effects against *Agrobacterium tumefaciens,* a notably difference was unregistered. Dilution method showed a resistance against all tested oils. But, the inhibition zones were lower than positive control for this strain. This difference could be attributed on the one hand to the inoculum's concentration, and on the other hand to the experimental conditions such as the diameter of inhibition area doesn't reveal the antibacterial efficacy of any component. This depends to the oil solubility and his diffusion in the agar.

The antifungal efficacy of volatiles against six fungal strains was determined. The main antifungal activity in the Arbequina cores volatiles, for all tested fungi, could be attributed, in part, to the raised level of aldehydes, such as nonanal, (E)-2-decenal (46.9% of total volatile compounds). This result was in accordance with Batinellia et al. [2006] who suggested that some aliphatic aldehydes from olive fruit volatile revealed an important antifungal activity against tested fungi. Zhang et al. [2017] proved the antifungal activity of nonanal, in tomato fruits, against *Penicillium cyclopium*. These authors exhibited that the nonanal showed a very strong inhibitory activity against the mycelia growth of *P. cyclopium* by severe disruption of the fungal cell membrane resulting in leakage of potassium ions and cell contents, and increasing total lipid content, extracellular pH and membrane permeability.

The antifungal activity and ACP analysis showed that Koroneiki pulp volatiles was more active against fungal species than their core oils, which exhibited an activity only against *Neofusicoccum sp, Phytophthora* sp. and *Trichoderma harzianum*. This activity could be attributed to the richness of the pulps in geranic acid and camphor. According to Yang et al.

Table 8. Person's correlation between the volatile compounds content and the microbiological and antioxidants activities from cores and pulps of three olive tree cultivars cultivated in an open field: Chemlali, Koroneiki and Arbequina

	Lim	MD	Nonanal	Camphor	Dodecene	Decanal	Decen	DCZ	DCE	Ger A	Tcene	Tetrad	(E,E)-α--farnesene	Hexa-decene	Hexa-decane
Limonene (Lim)	1														
Methyldecane (MD)	0.56**	1													
Nonanal (NL)	0.27**	-0.40**	1												
Camphor (CAM)	0.64**	0.69**	0.36**	1											
Dodecene (Dod)	0.58**	0.99**	-0.41**	0.65**	1										
Decanal	0.61**	0.93**	-0.43**	0.54**	0.97**	1									
E-2-decenal (Decen)	0.34**	-0.20**	0.00ns	-0.35**	-0.18**	-0.12**	1								
(E,Z)- decadienal	-0.20**	-0.62**	0.64**	-0.18**	-0.61**	-0.57**	0.05**	1							
(E,E)-2,4-decadienal	-0.10**	-0.53**	0.52**	-0.21**	-0.50**	-0.42**	0.04**	0.97**	1						
Geranic acid	0.20**	0.74**	-0.18**	0.71**	0.66**	0.45**	-0.26**	-0.47**	-0.52**	1					
Tetradecene	0.74**	0.23**	0.72**	0.74**	0.20**	0.11**	0.23**	0.14**	0.04**	0.36**	1				
Tetradecane	0.55**	1.00**	-0.39**	0.70**	0.98**	0.91**	-0.21**	-0.62**	-0.54**	0.77**	0.24**	1			
(E,E)-α-farnesene	0.30**	0.58**	-0.53**	0.10**	0.65**	0.79**	-0.13**	-0.62**	-0.45**	-0.06**	-0.27**	0.5**	1		
Hexadecene	0.80**	0.26**	0.69**	0.72**	0.24**	0.20**	0.28**	0.13**	0.06**	0.27**	0.99**	0.26**	-0.1ns	1	
Hexadecane	0.50ns	1.00**	-0.39**	0.71**	0.98**	0.91**	-0.21**	-0.62**	-0.54**	0.78**	0.25**	1.00**	0.53**	0.26**	1
IC_{50}ABTS	-0.3**	**-0.75****	0.27**	**-0.51****	**-0.74****	**-0.68****	0.22**	0.03**	-0.08**	**-0.60****	-0.12*	**-0.75****	-0.2*	-0.1**	-0.75**
IC_{50}DPPH	**-0.6****	**-0.29****	-0.30**	**-0.51****	**-0.27****	-0.21**	-0.36**	0.46**	0.56**	**-0.34****	-0.74**	**-0.30****	-0.1*	-0.74**	-0.30**
AF2 (anti-*Neofusicoccum*)	0.11**	0.34	-0.57**	-0.08**	0.32**	0.26**	0.42**	-0.85**	-0.88**	0.35**	-0.06*	0.34**	0.28**	-0.07**	0.35**
AF1 (anti-*Phytophtora*)	-0.20**	0.06	-0.36**	-0.12**	0.00ns	-0.14**	0.27**	-0.64**	-0.77**	0.41**	-0.03**	0.09**	-0.2**	-0.12**	0.09**
AF3 (anti-*Trichoderma*)	0.00**	-0.11	0.27**	0.21**	-0.18**	-0.34**	0.17**	-0.33**	-0.53**	0.37**	0.45**	-0.07*	-0.4**	0.35**	-0.06**
AF4 (anti-*Aspergellus*)	0.20**	-0.31**	0.02ns	-0.41**	-0.32**	-0.31**	0.97**	0.08**	0.03**	-0.20**	0.20**	-0.31**	-0.3**	0.20**	-0.31**
AF6 (anti-*Botritys*)	0.40**	-0.49**	**0.79****	0.03**	-0.50**	-0.49**	**0.60****	**0.51****	0.39**	-0.32**	**0.68****	-0.49**	-0.5**	**0.68****	-0.49**
AF5 (anti-*Fusarium*)	0.10**	-0.10**	-0.32**	-0.54**	-0.04**	0.10**	0.70**	0.23**	0.36**	-0.44**	-0.25**	-0.13**	0.15**	-0.16**	-0.14**
AB2 (anti-*Pseudonomas*)	0.00**	**0.66****	-0.74**	0.09*	**0.71****	**0.78****	-0.43**	-0.58**	-0.40**	0.19**	-0.51**	**0.64****	**0.85****	-0.43**	**0.63****
AB1 (anti-*Agrobacterium*)	-0.50**	-0.67**	**0.61****	-0.11**	-0.68**	-0.66**	-0.52**	**0.59****	**0.51****	-0.43**	-0.06*	-0.66**	-0.4**	-0.11**	-0.66**

Marked correlations are significant at: *p < 0.01, **p > 0.001, ns: no significant > 0.05

Lim – Limonene; MD – Methyldecane; NL – Nonanal; Decen – E-2-decenal; DCZ – (E,Z)-decadienal; DCE – (E,Z)-decadienal ; Ger A – Geranic acid; Tcene – Tetradecene; Tetrad – Tetradecane

[2011] the geranic acid inhibits mainly the activity of both fungi, *Colletotrichum graminicola* and *Fusarium graminearum*. The camphor was also used to treat fungal infections [Fahmidabinti et al. 2016]. In addition, according to disc diffusion tests the Chemlali pulps volatiles showed moderate to low antifungal activity against *Neofusicoccum* sp., *Phytophthora* sp., *Botritys* and *Trichoderma*. This activity could be attributed on the one hand to the synergistic effect between all volatile compounds [Gill et al. 2002]; and on the other hand, to the interaction between important and minor components and accomplished by their activity [Kalemba and Kunicka 2003, Yu et al. 2004].

The antioxidant capacity of the pulp and core fruit volatiles of the three *Olea europaea* L. cultivars has been determined by DPPH and $ABTS^{\bullet+}$ tests. The antioxidant activities of volatilesin all samples were directly proportional to the applied amount, the cultivars, the tested organs and the time of the reaction. The pulp volatiles of three cultivars showed higher antioxidant capacity than the core ones for scavenging radicals. In fact, the Koroneiki pulp volatiles showed the highest scavenging ability on DPPH and $ABTS^{\bullet+}$ free radicals than the other organs (with an IC50 value of 1.3 $mg \cdot mL^{-1}$). The ACP analysis showed a high relationship between antioxidants properties of volatiles and the limonene, methyldecane, dodecene, decanal, camphor, geranic acid, tetradecene, hexadecane and tetradecane levels in organs.

This difference on the antioxidant activity could be attributed essentially to the cultivar and to the quantitative difference of major and minor active components [Brahmi et al. 2011], such as linalool, camphor and α-farnesene which showed a strong capacities to scavenging free radicals [Ruberto et al. 2000, Ruberto and Baratta 2000, Wang et al. 2008, Zhang et al. 2010]. This result was in accordance with those of Wang et al. [2008] which suggested the capacity of the essential oil to scavenging free radicals is the cooperating results of their compositions. According to Miladi et al. [2013] a high relationship was observed between the volatiles and their antioxidant activity and their content in oxygenated monoterpenes. Ruberto and Baratta [2000] approved a high antioxidant activity of the camphor, in about a hundred of tested essential oils.

CONCLUSION

Results confirm that volatile compounds content in the fruit depends to the varieties and the organs. However, for three varieties the cores showed richness in aldehydes higher than the pulps especially (E)-2-decenal and nonanal. As far as, the studied varieties are concerned, we noticed that the cultivar is the most important factor influencing the antioxidant activity of the olive. The two tests, DPPH and ABTS, confirmed that the volatiles extracted from Arbequina cores and Koroneiki pulps are the most powerful to scavenging free radicals. In so far as,are concernedthe antimicrobial and antifungal activities, results showed that the studied samples presented an important differences in their activities. The oils extracted from the variety Chemlali, pulps and cores, have been shown to be effective antibacterial agents against *Pseudomonas savastanoi* pv savastanoi. Also, the Koroneiki pulp volatiles explained antifungal properties more active against *Neofusicoccum* sp., *Phytophthora* sp. and *Trichoderma harzianum* and Arbequina cores against *Botritys*. Morover, the essential oils extracted from Arbequina cores and Koroneiki pulps could be attributed a good fungistats

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