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Characterization of the Volatile Constituents in the Essential Oil of *Pistacia lentiscus* L. from Different Origins and Its Antifungal and Antioxidant Activity

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Essential oil (EO) from aerial parts (leaves, juvenile branches, and flowers when present) of *Pistacia lentiscus* L. growing wild in five localities of Sardinia (Italy) was extracted by steam-distillation (SD) and analyzed by gas chromatography (GC), FID, and GC-ion trap mass spectrometry (ITMS). Samples of *P. lentiscus* L. were harvested between April and October to study the seasonal chemical variability of the EO. A total of 45 compounds accounting for 97.5−98.4% of the total EO were identified, and the major compounds were α-pinene (14.8−22.6%), β-myrcene (1−19.4%), *p*-cymene (1.6−16.2%), and terpinen-4-ol (14.2−28.3%). The yields of EO (v/dry w) ranged between 0.09 and 0.32%. Similar content of the major compounds was found in samples from different origins and seasonal variability was also observed. The EOs were tested for their antifungal activity against *Aspergillus flavus*, *Rhizoctonia solani*, *Penicillium commune*, *Fusarium oxysporum*. Two samples were weakly effective against *Aspergillus flavus*. Furthermore, terpinenol and α-terpineol, two of the major components of EO of *Pistacia lentiscus* L., totally inhibited the mycelian growth of *A. flavus*. Quite good antioxidant activity of the EO was also found.

KEYWORDS: ***Pistacia lentiscus* L.; essential oil; harvesting time; antifungal activity; antioxidant activity**

INTRODUCTION

Pistacia lentiscus L. (mastic tree) is an evergreen bush belonging to the genus *Pistacia* of the Anacardiaceae family, which includes numerous wild and cultivated species, distributed in Mediterranean countries and Middle Eastern areas (*1*). In Italy, *P. lentiscus*, *P. vera*, and *P. terebinthus* are the most widespread species (*2*). In Sardinia (Italy), it grows along the coast from sea level to 700 meters above sea level (*1*). Different plant extracts are obtained from *P. lentiscus*, whereas the three main products are the resin trunk exudates, called "mastic gum", the cold pressed oil extracted from the berries, and the essential oil (EO) from the flowers, juvenile leaves, and branches and from the mastic gum. The extracts obtained from the mastic tree have been used since antiquity in traditional medicine, mainly as anti-inflammatory, antiseptic, and in the treatment of various diseases such as gastralgia and dyspepsia (*3*). Hepatoproctetive activity of the aqueous extract was reported (*4*). Recently, antioxidant property was evidenced (*5*), and the use of the extracts in cosmetic products was also reported (*6*). Moreover, the cold pressed oil from the mature berries has been used as olive oil substitute in food preparation (*3*). The composition, antimicrobial, and antibacterial activity of the EO from leaves and branches has been widely studied (*7*−*13*). The insecticidal and repellent activity of the EO was reported by Lamiri et al. (*14*), and Pascual-Villalobos and Robledo (*15*), respectively, whereas Duru et al. (*16*) and Kordali et al. (*17*) reported on the antifungal activity of the EO and the resin, respectively. Andrikopolous et al. (*18*) reported on the low-density lipoprotein (LDL) antioxidant activity of the hydrodistilled EO and of the resin. Pertaining to chemical composition of the EO of the Sardinian *P. lentiscus* L., Congiu et al. (*12*) reported the difference between hydrodistillation and supercritical fluid extraction (SFE) of the EO. Zrira et al. (*8*) reported on the influence of the season, the part of the plant, and the site of harvest on the EO composition and yields of Moroccan *P. lentiscus* L. Similar evidence was found for other Sardinian officinal plants by our research group (*19*, *20*).

To our knowledge, no investigations were performed on the chemical composition variability related to the season and geographical origin from samples of *P. lentiscus* L. collected in Sardinia or other Italian region, and only one paper (*12*) was found on the characterization of the chemical composition of the EO of this plant collected in south Sardinia. In this study we deeply examine the EO compositions and yields from aerial parts (leaves, juvenile branches, and flowers when present) of

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Pistacia lentiscus L. growing wild in five different areas of Sardinia, the chemical variation of EO profiles in different vegetative stages of the plant, and the antifungal activity of the EO against common plant fungi. The antioxidant activity is also reported.

MATERIALS AND METHODS

Plant Material. Random plant samples (RPS) of about 4 kg of aerial parts (leaves, juvenile branches, and flowers when present) *P. lentiscus* L. were collected early in the morning from five natural stations in Sardinia (Italy) of almost 3000 m^2 [Torre delle Stelle (TDS), Villaputzu (VLP), Orroli (ORR), Oristano (ORS), and Alghero (ALG)] at four different vegetative stages: full blooming ((1) April), end of blooming ((2) May), and growth of juvenile branches ((3) July, and (4) October). After harvesting, the samples were carried, in jute bags at about 20 °C, to the laboratory and immediately processed and analyzed. A total of 10 g of each sample was left to dry at 100 °C for 1 h to assess the water content. The specimens were identified and deposited in the Herbarium of the Department of Toxicology of the University of Cagliari, Italy. Each experiment was carried out in triplicate.

Chemicals. α-Pinene, camphene, β-pinene, myrcene, α-terpinene, p-cymene, γ-terpinene, α-terpinolene, borneol, terpinen-4-ol, α-terpineol, bornyl-acetate, β-caryophyllene, and *allo*-aromadendrene were obtained from Aldrich, Acros, and Fluka, (Milan, Italy); α-thujene, sabinene, limonene, linalool, α-humulene, methyl-eugenol, and α-phellandrene were analytical standard grade purchased from Extrasynthese (Genay, France). *n*-Hexane and ethyl acetate were analytical grade solvents purchased from Carlo Erba (Milan, Italy), and Na_2SO_4 was analytical reagent grade (Carlo Erba, Milan, Italy). DPPH (1,1-diphenyl-2-picryl-hydrazyl) and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were analytical standard grade (Sigma-Aldrich, Milan, Italy). Potato dextrose agar (PDA) was Oxoid L.T.D. (Basingstoke, Hampshire, England) and Parafilm "M" was American National Can (Chicago, IL). Water was distilled and filtered through a Milli-Q apparatus (Millipore, Milan, Italy).

Distillation. A total of 100 g of leaves and juvenile branches, and/or flowers (when present), were collected from the homogeneous RPS. Three samples were hydro-distilled simultaneously for 2 h in a Clevenger-type apparatus to assess the yield according to the Italian Official Pharmacopoeia XI (2002) (*21*). The EO was recovered directly using a micropipette from the upper part of the distillate without adding any solvent. EO composition analysis was carried out on samples obtained after steam-distillation of leaves and juvenile branches, and/or flowers (when present), for 4 h using a semi-industrial stainless steal apparatus of 80 liters capacity. The EOs were stored with anhydrous sodium sulfate in dark vials at 4 °C. EO solutions at 0.5% (v/v) were prepared in *n*-hexane before gas-chromatographic analysis.

GC/FID Analysis. A gas chromatograph Trace (Thermo Finningan, Rodano, Milan, Italy) equipped with a FID detector, an AS 800 auto sampler, and a split-splitless injector was used.

The capillary column was a fused silica DB5 (5% phenylmethylpolysyloxane, 30 m, 0.25 mm id; 0.25 μm film thickness; J&W Scientific, Folsom, CA). The injector and the detector were operated at 250 °C and 280 °C, respectively. A 1 μL aliquot of sample was injected in split mode (1:35). The oven was programmed as follows: 60 °C, raised to 65 °C (1 °C/min), raised to 120 °C (3 °C/min), raised to 180 °C (5 °C/min) and isothermally held for 5 min, raised to 250 °C (5 °C/min) and isothermally held for 20 min. Helium (purity 99.999%) and nitrogen at 120 Kpa and 80 Kpa, respectively, were used as carrier and make-up gases. H_2 and air were used at 150 and 100 Kpa, respectively.

GC/ITMS Analysis. A Varian CP 3800 gas chromatograph (Varian, Inc., Palo Alto, CA) coupled with a Saturn 2000 ion trap mass selective detector (ITMS), a Varian CP 7800 autosampler, a split-splitless injector, and a Saturn GC/MS Workstation 5.41 was used.

The column was a fused silica capillary DB-5MS (5% phenylmethylpolysyloxane, 30 m × 0.25 mm; film thickness 0.25 μm; J&W Scientific, Folsom, CA). Trap, manifold, and transfer line temperatures were set at 170, 100, and 200 °C, respectively. The mass spectrometer was calibrated weekly following the instrument software autotune test. The oven temperature was programmed the same as the GC/FID oven. Helium was used as carrier gas at 1 mL/min; 1 μL of sample was injected in split mode (1:35). MS conditions were as follows: ionization mode EI from 50 to 450 amu, multiplier voltage (1×10^5 gain) of 1400 V, multiplier offset +100. The EO components were identified by comparison of their relative retention times with those of authentic standard references, using computer matching against commercial library (*22*, *23*) and homemade library mass spectra from pure substances and components from known oils. Kovats indexes (KI) were collected and compared with those from mass-spectrometry literature data (*22*). Quantitative analysis of oil component percentages was carried out by peak area normalization measurement.

Antioxidant Activity. The antioxidant activity was determined by DPPH spectrophotometric test. DPPH assays were carried out according to Brand-Williams et al. (*24*). Results were expressed as Trolox equivalent antioxidant capacity (TEAC) in mmol of Trolox/L. A total of 20 μL of sample or standard solution at different concentrations (0.2, 0.4, 1.0, 2.0, and 4.0 mM) was added to 2 mL of DPPH (40 μM in ethyl acetate) and the mixture was shaken by hand. EO (10 μL) was dissolved in 3 mL of ethyl acetate. After 1 h of incubation in the dark and at room temperature, the absorbance of the samples was read versus the blank at λ of 517 nm using a Cary 50 spectrophotometer (Varian, Milan, Italy).

Antifungal Assay. The agar disk diffusion method was used. *Fusarium oxysporum*, *Rhizoctonia solani*, *Aspergillus flavus*, and *Pennicillium commune* were the fungi used. Fungi were isolated and identified from feeds in the Laboratory of Hygiene of the University of Cagliari. Petri dishes containing PDA were inseminated with mycelia fragments of 6 mm in diameter (10 days hold). Three paper disks were placed on the PDA medium in each petri disk. Two experiments were carried out: (a) 60 μL of pure EOs and (b) 60 μL, 40 μL, or 20 μL of single major compounds (α-terpineol, terpinen-4-ol, α-phellandrene, terpinolene, γ-terpinene, and α-terpinene). The plates were sealed with Parafilm M to avoid air dispersion of the volatiles and incubated in the dark at 22 °C. Samples with the mycelia in PDA and paper disk impregnated with distilled water instead of EO were used as control. The effectiveness of the treatments was evaluated by measuring the average diametric growth of the colonies at 4, 8, and 12 days after the inoculation. The percentage of inhibition was calculated according to the following equation of Zygadlo et al. (*25*):

$$I = 100(C - T)C^{-1}$$

Table 1. Analysis of the Variability of the Chemical Composition of the EO from Single Plants of *Pistacia lentiscus* L,[a] Collected from the Same Stations

	ORR				TDS			
cmpd	1	2	3	RSD[b] %	1	2	3	RSD[b] %
α-pinene	19.5	11.5	10.8	34.7	7.5	7.9	10.9	21.2
camphene	3.1	2.1	1.3	41.6	1.3	0.4	3.3	89.1
sabinene	0.6	0.5	0.3	32.7	3.9	2.7	1.7	39.8
β-pinene	7.8	6.5	5.4	18.3	0.9	2.5	6.3	85.8
myrcene	1.3	1.0	1.4	16.9	1.1	0.9	0.9	11.9
α-phellandrene	7.2	3.1	4.3	43.3	10.8	2.8	4.7	68.5
α-terpinene	1.1	0.4	1.3	50.6	6.1	2.0	1.9	71,9
p-cymene	7.0	9.4	6.2	22.1	2.4	8.2	3.8	63.1
limonene	5.5	5.8	6.0	4.4	4.7	1.5	1.8	66.3
β-phellandrene	9.7	7.7	8.6	11.6	5.7	3.0	4.0	32.2
γ-terpinene	2.8	3.2	4.3	22.6	5.4	4.7	4.4	10.6
α-terpinolene	1.4	1.2	2.0	27.2	2.6	2.5	1.5	27.6
terpinen-4-ol	7.7	14.6	11.1	31.0	22.4	24.9	12.9	31.6
α-terpineol	3.6	7.0	4.2	36.8	2.2	5.0	3.9	38.1
β-caryophyllene	4,8	5,7	3,7	21,2	1,6	5,2	3,7	51.7
germacrene D	2.4	1.8	1.8	17.3	7.0	7.7	11.6	28.3
δ-cadinene	0.7	1.0	2.4	66.4	1.3	3.4	3.3	44.4
total	86.2	82.5	75.1		86.9	85.3	80.6	

[a] Analyses have been performed on the EO obtained from about 4 Kg of three single plants. [b] Relative standard deviation; ORR: Orroli; TDS: Torre delle Stelle.

where I = inhibition, C = average diameter of fungi grown in PDA + water, and T = average diameter of fungi cultivated in PDA + EO.

All the experiments were replicated three times and the standard error was calculated.

Statistical Analysis. Statistical analyses were performed by GenStat v. 7.1 software (VSN International Ltd., Herts, U.K.) statistical program. Analysis of variance (ANOVA) was carried out according to a single factor, complete randomized block design with three replicates for each treatment. Mean comparisons of the effects of treatments were calculated, when applicable, by the Tuckey post hoc test, $p \leq 0.05$.

RESULTS AND DISCUSSION

Moisture Content and Essential Oil Yields. EO yields averaged 0.17% with a RSD (relative standard deviation) max of 33%. The moisture content during the experiment was not affected by seasonal variation and averaged 48%, with RSD max of 12%, with the lowest values for ORR in July (36%) and the highest for ALG in October (60%). The soils of ORR and ORS were subacid, with pH values of 6.4, those from ALG and VLL were sub-basic, with pH of 8.5, and that from TDS was almost neutral (pH 7.5).

Chemical Analysis. A total of 45 compounds were identified, accounting for 97.5% to 98.36% of the total compounds in the EO. The main compound in all samples was α-pinene, followed by terpinen-4-ol and *p*-cymene. The chemical variability between the EO from plants grown in the same station was assessed, analyzing samples collected from single plants at the end of blooming (period 2; Table 1). Two stations (ORR and TDS) were investigated. A total of 17 compounds accounting for more than 80% of the EO were selected. The analysis showed high RSD, denoting a huge difference in the concentration of the single compounds of the EO. These data agree with similar experiments carried out on *Helichrysum italicum* ssp. *microphyllum* (*19*) requiring the use of representative samples collected randomly from large areas to assess the true composition of the EO from a particular area.

Table 2 reports the chemical analysis of the EO from the five stations studied at full flowering stage (period 1) of the plant and includes all compounds identified in the EO of *P. lentiscus* L. The analysis of the table showed high variability of the single compounds among the EO from the distinct stations, which preclude the identification of different chemotypes. To simplify the discussion of the table, only compounds with more than 5% abundance were selected. TDS and ALG were characterized by four compounds, α-pinene, *p*-cymene, and terpinen-4ol, plus *β*-myrcene in TDS and sabinene, in ALG. ORR was typified by the five compounds, α-pinene, *β*-pinene, *β*-myrcene, *p*-cymene, and terpinen-4ol. VLP and ORS were distinguished by six compounds, α-pinene, α-terpinene, and terpinen-4-ol, plus *β*-myrcene, *γ*-terpinene, and germacrene D in VLP and α-phellandrene, *β*-phellandrene, and *p*-cymene in ORS. The data presented here are not in agreement with those from Congiu et al. (*12*) who reported the composition of *P. lentiscus* L. from a Sardinian southeast area (Costa Rey). The sample was characterized by *β*-pinene (18.7%), *β*-caryophyllene (13.2%), *β*-phellandrene (12.6%), and camphene (8.7%) as major compounds. Minor compounds were α-pinene, myrcene, and terpinen-4-ol, whereas these are the major ones in the southeast sample (VLP) analyzed in this paper. Nevertheless, germacrene D was found to be in a similar amount (3.91% Costa Rey and 5.06% VLP). Boelens and Jimenez (*9*) reported *β*-myrcene (19.25%) as the major compound of the *P. lentiscus* L. EO from Spain, followed by α-pinene (11.2%), *β*-caryophyllene (8.6%), terpinen-4-ol (8.41%), and germacrene D (6.35%). Germacrene D was detected at an average value of 3% or trace in samples from TDS and ALG analyzed in the present study, while *β*-caryophyllene was 1.5% on average.

Terpinen-4-ol was the major compound of the EO of *P. lentiscus* L. from two Moroccan localities, Chaouen (38.25%) and Media (16.9%) (*8*), but was not detected in the oil from Oulmes (Morocco), where the major compounds were α-pinene (27.5%), followed by myrcene (10.85%), and limonene (8.3%). EO from Chaouen (Morocco) has been found to possess noticeable amounts of α-pinene (10.3%), bornyl-acetate (8.55%), *β*-caryophyllene (4.75%), and myrcene (4.45%), which were minor compounds in the EO from Media (Morocco). Sabinene (4.7%), limonene (7.4%), and *γ*-terpinene (5.55%) were also found in this latter sample. Peculiar chemotypes were the EO of Turkish *P. lentiscus* L. (*16*), characterized by α-pinene (21.7%) and *β*-pinene (38.7%), whereas the oil from Egypt (*10*) was *δ*-3-carene rich (65.3%), never detected in Mediterranean oils except in that from Chaouen (Morocco) (*8*).

P. lentiscus L. from Corsica (*7*) was divided in three principal groups depending on the geographical origin. The first was α-pinene/terpinen-4-ol chemotype, the second was terpinen-4-ol/limonene chemotype, and the third was myrcene-rich (88%). Our samples were closest to the first Corsican chemotype, α-pinene/terpinen-4-ol chemotype.

Chemical Variability. A total of 13 compounds listed in Table 3, which accounted for >70% of the EO, except for TDS-4, ORR-2 and -4, and VLP-4 (ranging from 60 and 70%), were selected to discuss the seasonal variability of the oils from different geographical origin. Table 3 evidenced the fluctuation in the pathway of the *a*-terpenyl-catione intermediate (*26*), depending on the vegetative stage of the plants. Samples from TDS, ORR, VLP, and ORS showed similar trends for all compounds, respectively. Sabinene remained almost constant during the experiment, but some compounds varied strongly. Particularly, α-phellandrene, α-terpinene, *γ*-terpinene, and α-terpinolene increased, whereas *p*-cymene and limonene

Table 2. Chemical Composition (Area %) of the Essential Oil of the Aerial Parts of *Pistacia lentiscus* L. at Full Flowering Stage

cmpd	KI[a]	LRI[b]	identification methods[c]	harvesting area				
				TDS[d]	ORR[d]	VLP[d]	ORS[d]	ALG[d]
tricyclene[e]	921	920	MS, RI	0.90	0.48	0.20	0.41	0.36
α-tujene	924	924	MS, RI, std	0.15	0.45	0.21	0.64	0.45
α-pinene	931	932	MS, RI, std	20.40	19.15	14.81	21.55	22.59
camphene	947	946	MS, RI, std	3.60	2.31	1.05	1.85	1.59
sabinene	970	968	MS, RI, std	2.51	1.17	3.67	3.88	8.13
β-pinene	976	979	MS, RI, std	3.46	5.50	2.62	4.32	3.62
β-myrcene	988	991	MS, RI, std	18.29	19.36	10.44	1.40	0.99
α-phellandrene	1002	1003	MS, RI, std	0.20	0.62	4.91	10.84	0.06
α-terpinene	1015	1017	MS, RI, std	t[f]	0.30	5.12	5.59	t
p-cymene	1024	1025	MS, RI, std	14.79	9.92	1.59	2.17	16.22
limonene	1027	1029	MS, RI, std	3.03	3.76	0.93	1.83	2.23
β-phellandrene[e]	1029	1030	MS, RI	2.88	3.80	3.09	5.39	2.35
γ-terpinene	1056	1060	MS, RI, std	0.57	1.32	6.56	5.15	0.29
α-terpinolene	1083	1080	MS, RI, std	0.13	0.40	2.04	2.40	t
2-nonanone[e]	1091	1090	MS, RI	0.66	1.24	0.44	0.51	0.37
linalool	1100	1097	MS, RI, std	0.19	t	t	0.19	0.17
α-fencocanphone[e]	1103	1106	MS, RI	0.20	0.31	t	0.12	t
isoamyl isovalerate[e]	1107	1103	MS, RI	t	0.23	t	t	t
menth-2-en-1-ol-*cis*-para[e]	1123	1122	MS, RI	t	t	0.19	0.18	0.45
menth-2-en-1-ol-*trans*-para[e]	1141	1141	MS, RI	t	t	0.11	t	0.29
borneol	1170	1169	MS, RI, std	0.06	0.14	0.07	t	t
terpinen-4-ol	1179	1177	MS, RI, std	14.17	15.07	19.74	19.84	28.29
α-terpineol	1193	1189	MS, RI, std	1.93	3.24	2.64	1.80	2.67
ascaridole[e]	1242	1237	MS, RI	0.21	t	t	t	t
carvone oxide *cis*[e]	1252	1263	MS, RI	1.05	0.38	t	0.08	1.72
ascaridole glicol *trans*[e]	1273	1269	MS, RI	0.11	t	t	t	0.21
bornyl acetate	1285	1289	MS, RI, std	2.24	0.74	0.67	0.51	0.72
2-undecanone[e]	1292	1292	MS, RI	0.98	1.58	0.78	1.01	0.43
4-hydroxy-crypton[e]	1319	1316	MS, RI	0.28	t	t	t	0.13
δ-elemene[e]	1348	1338	MS, RI	0.64	0.19	0.41	0.36	t
α-copaene[e]	1374	1377	MS, RI	t	0.19	0.41	t	t
β-elemene[e]	1387	1391	MS, RI	t	t	0.24	0.15	t
methyl eugenol	1398	1404	MS, RI, std	t	t	t	t	0.18
β-caryophyllene	1415	1419	MS, RI, std	1.30	2.29	1.74	1.23	0.89
2-methylbutyl benzoate[e]	1437	1441	MS, RI	0.21	t	0.21	0.12	0.23
α-humulene	1453	1450	MS, RI, std	0.46	0.57	0.90	0.45	0.46
aromdendrene-*allo*	1462	1461	MS, RI, std	t	t	0.29	t	t
muurola-4-(14),5-diene *cis*[e]	1470	1467	MS, RI	t	t	0.27	0.17	t
cadina-1(6),4-diene *trans*[e]	1473	1477	MS, RI	0.51	0.39	0.63	0.29	0.60
germacrene D[e]	1478	1480	MS, RI	t	1.71	5.06	2.11	t
γ-amorfene[e]	1495	1496	MS, RI	0.51	0.41	t	t	0.50
δ-cadinene[e]	1524	1523	MS, RI	1.13	1.06	2.71	1.54	0.81
calamenene *trans*[e]	1532	1529	MS, RI	0.23	t	t	t	t
cubenol	1647	1647	MS, RI, std	t	t	1.01	t	0.36
valerianol[e]	1658	1658	MS, RI	t	t	1.74	t	t
total identified				97.98	98.28	97.5	98.08	98.36

[a] Kovats indexes (DB-5MS column). [b] LRI literature reported RI. [c] Methods of identification: MS, comparison with mass spectrum of computer mass libraries; RI, comparison with those from literature; and std, injection of authentic sample. [d] TDS: Torre delle Stelle; ORR: Orroli; VLP: Villaputzu; ORS: Oristano; ALG: Alghero. [e] Compound tentatively identified according to the mass spectrum (MS) and by comparison of RI with the literature (RI). [f] t = trace (<0.05%).

decreased in the oils isolated from TDS and ORR areas. These compounds seemed to be more stable in the oils from VLP and ORS, except for α- and β-phellandrene, which decreased at the end of the experiment. The sample from ORS-4 (Table 3) was characterized by a major amount of α-phellandrene, γ-terpinene, and limonene. Myrcene varied strongly during the experiment in the oils from TDS and ORR, whereas it was almost constant in the oils from VLP and ORS. Terpinen-4-ol decreased in all samples from the first to the fourth harvest. The sample from ALG was distinct from the other areas. Indeed, the trend followed by myrcene and α-pinene was closest to VLP and ORS, while the trends followed by the other compounds overlapped those of TDS and ORR.

This finding is in accord with recent results published by our research group (*20*) on the seasonal variability of *Lavanda stoechas* ssp. *stoechas* EOs. In contrast, Zrira et al. (*8*) reported the seasonal variability for *P. lentiscus* L. growing wild in Morocco, finding that generally the percentage composition of the volatiles remained stable during the harvesting time.

Antifungal Activity. All EO and single major compounds (α-terpineol, terpinen-4-ol, α-phellandrene, terpinolene, γ-terpinene, and α-terpinene) did not show activity against all fungi tested. Only the samples ORS-2 and ORS-3 exhibited low activity against *A. flavus*, and their effectiveness was reported in Table 4 (experiment a). Among the single compounds (experiment b), α-terpineol and terpinen-4-ol appeared to be effective, showing good activity also at the lowest concentration tested (20 μL). This could be attributed to the presence of the alcohol functional group, as it was already demonstrated by Griffin et al. (*27*) against *C. albicans*. Our data are not in agreement with Kordali et al. (*28*), who reported antifungal activity of α-terpineol and terpinen-4-ol against *R. solani* and *F. oxysporum*.

Table 3. Analysis of EO Major Compounds (area %) Composition of *Pistacia lentiscus* L. from Different Harvesting Stations in the Four Different Harvesting Period

	harvesting area[a]																			
	TDS				ORR				ALG				VLP				ORS			
	harvesting period[b]																			
cmpd	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
α-pinene	20.4	12.3	14.2	14.9	19.2	8.8	15.1	14.2	22.6	21.5	14.6	15.6	14.8	12.9	10.6	11.5	21.6	14.8	13.2	16.4
sabinene	2.5	2.5	1.5	2.4	1.2	0.4	1.7	2.7	8.1	4.2	4.6	3.8	3.7	2.6	2.8	4.1	3.9	3.5	2.5	4.6
β-pinene	3.5	4.2	2.8	4.0	5.5	4.0	3.9	3.8	3.6	3.9	2.3	3.2	2.6	2.6	1.3	1.6	4.3	2.7	2.3	4.3
myrcene	18.3	5.9	20.6	11.5	19.4	2.1	10.3	9.3	1.0	1.1	1.1	1.3	10.4	8.7	5.1	11.3	1.4	1.1	1.1	1.3
α-phellandrene	0.2	7.1	6.4	6.3	0.6	3.1	7.5	4.9	0.1	5.7	6.9	8.1	4.9	4.7	5.3	1.0	10.8	12.8	10.8	9.1
α-terpinene	t[c]	3.4	4.0	1.9	0.3	0.9	4.4	1.9	t	3.7	7.3	3.7	5.1	6.2	5.5	3.3	5.6	6.6	6.2	4.1
p-cymene	14.8	3.8	2.9	2.7	9.9	6.7	1.1	0.4	16.2	5.4	1.1	1.5	1.6	1.2	2.1	2.1	2.2	1.8	1.9	1.3
limonene	3.0	1.7	1.8	3.6	3.8	3.7	1.6	6.1	2.2	1.9	0.7	5.0	0.9	0.8	0.7	1.9	1.8	1.7	1.8	6.4
β-phellandrene	2.9	3.7	2.3	2.0	3.8	4.1	4.6	2.8	2.4	3.4	4.5	3.3	3.1	3.1	3.5	0.3	5.4	5.2	3.9	0.3
γ-terpinene	0.6	5.2	5.0	4.3	1.3	3.7	5.9	6.8	0.3	6.2	8.8	10.6	6.6	8.3	7.7	6.5	5.4	5.2	3.9	8.5
α-terpinolene	t	1.8	1.7	1.9	0.4	1.2	2.1	2.6	t	2.1	2.8	3.5	2.0	2.4	2.5	2.2	2.4	2.8	2.8	3.2
terpinen-4-ol	14.2	16.5	15.0	10.0	15.1	23.4	14.9	11.9	28.3	23.4	26.6	14.9	19.7	22.7	24.7	17.0	19.8	23.3	20.6	15.7
α-terpineol	1.9	3.1	3.1	5.0	3.2	4.4	4.3	6.2	2.7	3.4	3.5	4.3	2.6	3.3	3.9	4.0	1.8	2.3	3.7	3.8
total	80.4	68.1	78.2	65.5	80.5	62.1	73.1	67.4	84.8	82.5	81.3	74.5	75.4	76.2	71.8	62.7	84.6	81.5	71	75.2

[a] TDS: Torre delle Stelle; ORR: Orroli; VLP: Villaputzu; ORS: Oristano; ALG: Alghero. [b] 1 = full blooming (April); 2 = end of blooming (May); 3 = growth of juvenile branches (July); 4 = growth of juvenile branches (October). [c] t = trace (<0.05%).

Table 4. Inhibition of Mycelial Growth (Percent of Control)[a]

sample or cmpd tested	20 μL	60 μL
ORS[b] (2)	n.t.[c]	6.4[A d]
ORS[b] (3)	n.t.[c]	8.3[A d]
α-phellandrene	0[e]	0
α-terpinolene	0	0
α-terpineol	100[f]	100[B d]
terpinen-4-ol	100	100[B d]
γ-terpinene	0	0
α-terpinene	0	0
control	0	0

[a] Growth of fungal specie is given as mean of three replicates, with 60 μL of pure EO of *P. lentiscus* (experiment a) and different concentrations of the single major compounds against *Aspergillus flavus* (experiment b). [b] ORS: Oristano.[c] n.t. = not tested. [d] Values within a column for each experiment having different capitals letters are significantly different from each other, using Tukey's LDS test ($P < 0.05$). [e] 0 = full growth. [f] 100 = no growth.

Table 5. Antioxidant Activity (TEAC[a], mmol/L) of the EO of *Pistacia lentiscus* L.

	mean				
harvesting period[b]	TDS[c]	ORR[c]	ORS[c]	VLP[c]	ALG[c]
1	1.92 aA	2.58aB	0.52aC	0.52aC	2.78aB
2	1.66 aA	2.34aB	0.61aC	0.69bC	0.69bC
3	4.61bD	0.88 bC	0.68aC	1.40cA	2.45aB
4	3.09cD	4.41cC	0.93bC	0.92bC	1.39cA

[a] TEAC (Trolox equivalent antioxidant capacity). [b] 1 = full blooming (April); 2 = end of blooming (May); 3 = growth of juvenile branches (July); 4 = growth of juvenile branches (October). [c] TDS: Torre delle Stelle; ORR: Orroli; VLP: Villaputzu; ORS: Oristano; ALG: Alghero. Lower case letters indicate comparison in the column (different periods). Capital letters indicate comparison in the lines (different geographical origin). Data marked with different letters are significantly different from each other, using Tukey's LDS test ($P < 0.05$).

Antioxidant Activity. The antioxidant activity of the EO of *P. lentiscus* L. ranged between 0.52 and 4.61 mmol/L, with the highest values for TDS-3 and ORR-4 and the lowest for ORS-1, -2, and -3 and VLP-1 (Table 5). The high antioxidant activity variability among samples from different origin could not positively related to the chemical composition of the corresponding EO. The different trend followed by the EO compounds, as it is reported above, could justify the antioxidant variability, but it is difficult to relate these changes to one single compounds. Recently, Tuberoso et al. (*29*) reported the antioxidant activity of the EO from *Achillea ligustica*, all together with the antiradical activity of various EO from common aromatic plants, finding that *Rosmarinus officinalis* L. presented the lowest (0.17 mmol/L), where *Thymus capitatus* showed the highest (11.4 mmol/L). These data could be useful to give a measure of the antioxidant activity of the EO of *P. lentiscus* L. analyzed in this paper.

In conclusion, the data presented in this paper showed a high variability in the chemical composition of the EO from single plants of the same area, from random samples of different harvesting areas and vegetative stage. No final conclusion can be done to assess any genetic polymorphism of Sardinian *P. lentiscus* L., as it is known for *P. lentiscus* L. from some Mediterranean regions (*30*); hence, further investigation should be carried out to define the Sardinian genotype. Myrcene, α-phellandrene, α-terpinene, γ-terpinene, α-terpinolene, *p*-cymene, and limonene varied strongly depending on the vegetative stage of the plants from TDS and ORR. However, the antioxidant activity varied notably in the samples from the same area and of different harvesting periods, but it showed quite good values compared to the EO from other officinal plants. The EO showed low antifungal activity when applied at 60 μL/plate. Among the single major compounds, only terpinen-4-ol and α-terpineol showed good activity against *A. flavus*.

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