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## Variations in composition and antioxidant activity of Tunisian *Pistacia lentiscus* L. leaf essential oil

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### Abstract

In this study, the composition and antioxidant activity of *Pistacia lentiscus* L. leaf essential oil (EO) from four Tunisian localities were investigated. The EO yields (% w/w) of *P. lentiscus* leaf were 0.009% in Oued El Bir, 0.02% in Jebel Mansour, 0.007% in Siliana and 0.01% in Tabarka. The main compounds of Oued El Bir EO were terpinene-4-ol (41.24%) and  $\alpha$ -terpineol (7.31%), whereas those of Jebel Mansour were  $\alpha$ -pinene (9.48%), limonene (19.11%) and  $\alpha$ -phellandrene (3.20%). In Siliana samples, terpinene-4-ol (23.32%),  $\beta$ -caryophyllene (22.62%) and  $\alpha$ -terpineol (7.12%) were the main compounds. For *P. lentiscus* L. from Tabarka,  $\alpha$ -terpineol (9.79%) and  $\beta$ -caryophyllene (38.33%) were the major constituents. Three chemotypes of *P. lentiscus* EO were identified for the first time in Tunisia. *P. lentiscus* EOs were also screened for their antioxidant activities. The 1,1-diphenyl-2-picrylhydrazyl assay gives an  $IC_{50}$  range value of 60–110  $\mu$ g/mL for all the samples studied. EOs from different Tunisian localities showed lower  $\beta$ -carotene bleaching activity than butylated hydroxytoluene and butylated hydroxyanisol. *P. lentiscus* EO presents a very low capacity to chelate ferrous irons ( $IC_{50}$  = 80.8–104  $\mu$ g/mL) and no metal chelating activity was recorded.

**Keywords:** Tunisian *Pistacia lentiscus* L., essential oil, chemotypes, antioxidant activity,  $IC_{50}$

### Introduction

In recent years, the essential oil (EO) extracted from aromatic and medicinal plants and herbal extracts has attracted a great deal of scientific interest due to their potential as a source of natural antioxidants and biologically active compounds (Wannissorn et al. 2005; Hussaien et al. 2008; Basile et al. 2011; Minutolo et al. 2011). The use of plants and herbs as antioxidants in food, pharmaceutical and cosmetic industries is becoming of increasing importance as an alternative to synthetic antioxidants because their safety and efficacy are frequently questioned (Sokmen et al. 2004) and they have possible activity as promoters of carcinogenesis (Djeridane et al. 2006; Suhaj 2006).

Aromatic and medicinal plants as a source of natural antioxidants play a major role in the

prevention of various pathological conditions such as cancer, cardiovascular and neurodegenerative diseases believed to be associated with oxidative stress (Jiratanan & Liu 2004; Losso et al. 2007).

Reactive oxygen species (ROS) are highly reactive and cause damage to proteins, lipids, enzymes and DNA (Singh et al. 2009). In this context, many plant extracts and EOs are known to exert their effects via antioxidant mechanisms such as free-radical scavenging, chelating of pro-oxidant metal ions or quenching singlet oxygen formation (Valko et al. 2007; Lopes-Lutz et al. 2008).

*P. lentiscus* L. is an evergreen shrub belonging to the Anacardiaceae family characterized by a strong smell and green leaves (Arista et al. 1990). It is widely distributed in the Mediterranean and Middle Eastern area. In Tunisia, it occurs and grows especially in north

and north western mountains (Bonnier & Douin 1990).

*P. lentiscus* L. commonly called “Dharw” in Maghreb and “Mustaka” in the Middle East is employed in folk medicine against various pains. The aerial part of this plant has traditionally been used in some regions of Spain as a popular cure against hypertension (Villar et al. 1987), and its stimulant and diuretic properties were reported by Vidrich et al. (2004).

The first study concerning *P. lentiscus* L. dates back to 1966 on Corsica samples and indicated that its EO is rich in monoterpenes (Peyron 1966).

The resin obtained from *P. lentiscus* L. stem and the mastic gum are traditionally used by the healers to treat many diseases such as upper abdominal discomfort, stomach aches, dyspepsia and peptic ulcer (Al Said et al. 1986). Tassou and Nychas (1995) reported that the addition of mastic gum (*P. lentiscus* var. Chia) in broth culture inoculated with *Staphylococcus aureus*, *Lactobacillus plantarum*, *Pseudomonas fragi* and *Salmonella enteritidis* inhibited their growth. Investigations on *Pistacia* species also revealed that their crude extracts, EOs and some of their triterpenoid constituents exhibit anti-inflammatory, antifungal and antifeedant activities (Duru et al. 2003). Janakat and Al-Merie (2002) indicated that *P. lentiscus* L. aqueous extracts showed marked antihepatotoxic activity. Atmani et al. (2009) reported that the best overall antioxidant capacity was shown by methanolic extracts of *P. lentiscus* L. leaves followed by those of *Fractinus angustifolia* and *Climatis flamula*. Ballan et al. (2007) followed the anticancer activity and the apoptosis induction in human colon cancer cells and showed that Chios mastic gum (CMG) contains compounds that inhibit proliferation and induce death of HCT116 human colon cancer cells *in vitro*.

Many researchers studied the chemical composition of *P. lentiscus* L. EO from diverse origins in the aim to prospect eventual new chemotypes (Castola et al. 2000; Ben Douissa et al. 2005). Changes in the chemical composition of *P. lentiscus* L. leaf EO from different regions and bioclimatic floors harvested at different periods and extracted using different methods have been widely studied (Reverchon et al. 1993; Fernandez et al. 2000; Chryssavgi et al. 2008; Congiu et al. 2002). To the best of our knowledge, there are unpublished data about chemotypes from *P. lentiscus* EOs in Tunisia and only one paper dealing with the chemical composition of this EO species from one Tunisian site (Zaghouan) (Ben Douissa et al. 2005).

As part of our extensive screening programme of aromatic and medicinal plants from Tunisia, we report in this paper the EO compositions and antioxidant activities of wild populations of

*P. lentiscus*, collected from four distinct localities belonging to different bioclimatic floors, compared with other studies from different origins. The aim of this study was to investigate the variability of the *P. lentiscus* EO compositions and their antioxidant activities as affected by the region of collection.

## Materials and methods

### Chemicals

The solvent used in all the experiments was methanol purchased from Merck (Darmstadt, Germany). Butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA),  $\beta$ -carotene, linoleic acid, ethylenediaminetetraacetic acid (EDTA), 3-(2-pyridyl)-5,6-bis(4-phenyl-sulphonic acid)-1,2,4-triazine (ferrozine), iron (II) chloride tetrahydrate ( $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ ), iron (II) chloride ( $\text{FeCl}_2$ ), iron (III) chloride ( $\text{FeCl}_3$ ), 1,1-diphenyl-2-picrylhydrazyl (DPPH), polyvinylpyrrolidone, Folin–Ciocalteu reagent, potassium ferricyanide ( $\text{K}_3\text{Fe}(\text{CN})_6$ ) and aluminium chloride ( $\text{AlCl}_3$ ) were purchased from Sigma-Aldrich (Steinheim, Germany). These solutions were wrapped in aluminium foils and stored at 4°C. All other chemicals used were of analytical grade.

### Plant material

*P. lentiscus* L. leaves were collected from natural populations at the flowering stage during May 2008. Four localities were explored in north and north-western of Tunisia: Oued El Bir [latitude 36°45'18.45''(N), longitude 10°33'37.67''(E), altitude 50 m], Jebel Mansour [latitude 36°22'43.37''(N), longitude 9°59'14.64''(E), altitude 341 m], Siliana [latitude 36°4'55.56''(N), longitude 9°22'29.28''(E), altitude 427 m] and Tabarka [latitude 36°57'7.20''(N), longitude 8°45'29.16''(E), altitude 67 m]. These four sites belong to different bioclimatic floors as indicated in Table I.

The plant material was botanically characterized by Professor Smaoui (Borj Cedria Biotechnology Center, Tunisia) according to the morphological description presented in Tunisian flora (Pottier-Alapetite 1979). A voucher specimen (P.1.08003) was deposited in the Laboratory of Bioactive Substances (Biotechnology Center of Borj Cedria). The harvested material was air-dried at room temperature (20–25°C) for 2 weeks and stored in cloth bags.

### EO isolation

One hundred grams of air-dried aerial parts of *P. lentiscus* L. were subjected to hydrodistillation for 2 h in a simple laboratory Quik-fit apparatus that consisted of a 1000-mL steam generator flask,

Table I. *P. lentiscus* L. sampling sites and their bioclimatic characteristics.

	Bioclimatic floor	Average annual temperature (°C)	Average annual precipitation (mm)	Average annual humidity (%)
Oued El Bir	Subhumid	17 ± 1.2	41 ± 3.3	79 ± 6.6
Jebel Mansour	Semi-arid	16 ± 1.3	32.9 ± 3.0	75 ± 7.2
Siliana	Subhumid	14.5 ± 1.1	90.2 ± 7.5	76 ± 6.9
Tabarka	Humid	16 ± 1.4	58.2 ± 5.1	76 ± 7.5

Note: Data are means of three replicates ± SD.

a distillation flask, a condenser and a receiving vessel. A liquid–liquid extraction with diethyl ether and pentane v/v (Analytical Reagents, LabScan, Ltd, Dublin, Ireland) as solvent mixture permitted to recover EO. The organic layer was then concentrated at 35°C using a Vigreux column at the atmospheric pressure.

EOs were dried over anhydrous sodium sulphate and stored at 4°C until their analysis. In order to quantify EO and their main compounds, the 6-methyl-5-hepten-2-one (Sigma-Aldrich) was used as internal standard. All experiments were done in triplicates and the results were expressed on the basis of dry matter weight.

## Chromatographic analysis

### Gas chromatography

EOs were analysed by gas chromatography (GC) using a Hewlett-Packard 6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with a flame ionization detector (FID) and with an electronic pressure control injector. A polar HP Innowax (polyethylene glycol) column (30 m × 0.25 mm, 0.25 µm film thickness) and an apolar HP-5 column (30 m × 0.25 mm, 0.25 µm film thickness) from Agilent were used. The flow of the carrier gas (N<sub>2</sub>, U) was 1.6 mL/min and the split ratio was 60:1. Analyses were carried out using the following temperature programme: oven kept isothermally at 35°C for 10 min, increased from 35 to 205°C at the rate of 3°C/min and kept isothermally at 205°C for 10 min. Injector and detector temperatures were held, respectively, at 250 and 300°C.

### Gas chromatography–mass spectrometry

The gas chromatography–mass spectrometry (GC–MS) analyses were made using an HP 5972 mass spectrometer with electron impact ionization (70 eV) coupled with an HP 5890 series II gas chromatograph. An HP-5MS capillary column (30 m × 0.25 mm coated with 5% phenyl methyl silicone and 95% dimethyl polysiloxane, 0.25 µm film thickness) was used. The oven temperature was

programmed to rise from 50 to 240°C at a rate of 5°C/min. The transfer line temperature was 250°C. The carrier gas was helium with a flow rate of 1.2 mL/min; the split ratio was 60:1. Scan time and mass range were 1 s and 40–300 *m/z*, respectively.

### Compound identification

The volatile compounds of EOs were identified by comparing their retention index (RI) related to (C<sub>9</sub>–C<sub>18</sub>) *n*-alkanes with those available in the literature of authentic compounds (Analytical Reagents, LabScan, Ltd) available in the literature and in our laboratory and by matching their mass spectra fragmentation patterns with corresponding data stored in the mass spectra library of the GC–MS data system (National Institute of Standards and Technology) and other published mass spectra (Adams 2001). Relative percentage amounts of the identified compounds were obtained from the electronic integration of the FID peak area.

### Scavenging ability on DPPH radical

DPPH quenching ability of *P. lentiscus* EO from the four localities was measured by the bleaching of the purple-coloured solution of DPPH radical according to the method of Hanato et al. (1988). One millilitre of the extract at known concentrations was added to 0.25 mL of a DPPH methanolic solution. The mixture was shaken vigorously and left standing at room temperature for 30 min. The absorbance of the resulting solution was then measured at 517 nm after 30 min. The antiradical activity (three replicates per treatment) was expressed as IC<sub>50</sub> (µg/mL), the concentration required to cause a 50% DPPH inhibition. The ability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = \left[ \frac{A_0 - A_1}{A_0} \right] \times 100,$$

where  $A_0$  is the absorbance of the control at 30 min and  $A_1$  is the absorbance of the sample at 30 min. BHT was used as a positive control. Samples were analysed in triplicate.

### *β-Carotene bleaching test*

A modification of the method described by Koleva et al. (2002) was employed. β-Carotene (2 mg) was dissolved in 20 mL chloroform and to 4 mL of this solution, linoleic acid (40 mg) and Tween-40 (400 mg) were added. Chloroform was evaporated under vacuum at 40°C and 100 mL of oxygenated ultra-pure water was added; then the emulsion was vigorously shaken. Reference compounds (BHT and BHA) and sample EOs were prepared in methanol. The emulsion (3 mL) was added to a tube containing 0.2 mL of different concentrations of EOs. The absorbance was immediately measured at 470 nm and the test emulsion was incubated in a water bath at 50°C for 120 min, when the absorbance was measured again. BHT and BHA were used as positive control. In the negative control, the EO or the extract was substituted with an equal volume of methanol. The antioxidant activity (%) of the organ extracts and that of EOs were evaluated in terms of β-carotene bleaching using the following formula:

$$\% \text{ Inhibition} = \left[ \frac{A_t - C_t}{C_0 - C_t} \right] \times 100,$$

where  $A_t$  and  $C_t$  are the absorbance values measured for the test sample and control, respectively, after incubation for 120 min, and  $C_0$  is the absorbance value for the control measured at zero time during the incubation. Results are expressed as  $IC_{50}$  values ( $\mu\text{g/mL}$ ), the concentration required to cause a 50% β-carotene bleaching inhibition.

### *Chelating effect on ferrous ions*

The ferrous ion chelating activity of different EO samples was assessed as described by Zaho et al. (2006). Different concentrations of EOs prepared in methanol were added to 0.05 mL of  $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$  solution (2 mM) and left for incubation at room temperature for 5 min. Then, the reaction was initiated by adding 0.1 mL of ferrozine (5 mM), and the mixture was adjusted to 3 mL with deionized water, shaken vigorously and left standing at the room temperature for 10 min. Absorbance of the solution was then measured spectrophotometrically at 562 nm. The percentage of inhibition of ferrozine- $\text{Fe}^{2+}$  complex formation was calculated using the formula given below:

$$\text{Metal chelating effect (\%)} = \left[ \frac{A_0 - A_1}{A_0} \right] \times 100,$$

where  $A_0$  is the absorbance of the ferrozine- $\text{Fe}^{2+}$  complex and  $A_1$  is the absorbance of the test compound. Results were expressed as  $IC_{50}$ , efficient concentration corresponding to 50% ferrous iron

chelating. EDTA was used as a positive control. Samples were analysed in triplicate.

### *Iron reducing power*

The capacity of EOs to reduce  $\text{Fe}^{3+}$  was assessed by the method of Oyaizu (1986). One millilitre of different concentrations of EOs in methanol was mixed with 2.5 mL of a 0.2 M sodium phosphate buffer (pH 6.6, prepared from 62.5 mL of a 0.2 M  $\text{Na}_2\text{HPO}_4$  and 37.5 mL of 0.2 M  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ ) and 2.5 mL of 1%  $\text{K}_3\text{Fe}(\text{CN})_6$  and incubated in a water bath at 50°C for 20 min. Then, 2.5 mL of 10% trichloroacetic acid was added to the mixture that was centrifuged at 650 g for 10 min. The supernatant (2.5 mL) was then mixed with 2.5 mL distilled water and 0.5 mL of 0.1% ferric chloride solution. The intensity of the bluish-green colour was measured at 700 nm. The  $EC_{50}$  value (mg/mL) is the EO concentration at which the absorbance was 0.5 for the reducing power and was calculated from the graph of absorbance at 700 nm against the EO concentration. Ascorbic acid was used as a positive control.

### *Statistical analyses*

All experiments were carried out in triplicate and the results were presented as means of three repetitions  $\pm$  SD. The statistical analysis was carried out with STATISTICA (StatSoft 1998). Differences were tested for significance by using the ANOVA procedure using a significance level of  $P < 0.05$ .

## **Results and discussion**

### *EO yields*

EO yields obtained by hydrodistillation of *P. lentiscus* L. leaves are given in Figure 1. Significant differences ( $P < 0.05$ ) were observed in EO yields of the four localities which comprised between 0.009% and 0.02% (w/w on dry weight basis). Whereas Zrira et al. (2003) indicated that there are no differences between EO yields (0.2%) of leaves collected from Mehdia, Oulmes and Chaouen in Morocco. Our samples presented lower EO contents compared to those of Turkey (0.30%) (Duru et al. 2003), Spain (0.25%) (Fernandez et al. 2000) and Morocco (0.2%) (Zrira et al. 2003).

### *EO composition*

Analysis of *P. lentiscus* leaf EO, extracted during flowering stage from Oued El Bir, Jebel Mansour, Siliana and Tabarka, showed that 40 compounds

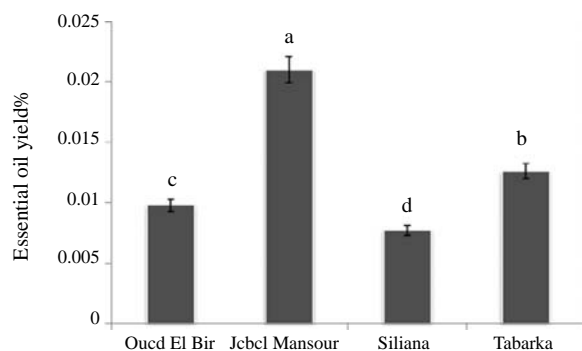


Figure 1. Yields (% on dry weight basis) of *P. lentiscus* L. leaf EOs from four Tunisian *P. lentiscus* L. localities. Values with different subscript (a–d) were significantly different at  $P < 0.05$  (Duncan test).

were identified which represent 73.53–91.18% of total constituents (Table II).

In Oued El Bir EO, the oxygenated monoterpenes had the highest contribution. This fraction was mainly composed of terpinene-4-ol (41.24%) and  $\alpha$ -terpineol (7.31%). Meanwhile, monoterpene hydrocarbons represented 2.64% and sesquiterpenes 3.87% of the total oil. The Oued El Bir chemotype was characterized by two major compounds: terpinene-4-ol and  $\alpha$ -terpineol. However, EO of plants collected from Jebel Mansour was rich in monoterpene hydrocarbons (45.12%) followed by oxygenated monoterpenes (19.38%) and sesquiterpenes (2.71%).  $\alpha$ -Pinene (9.48%), limonene (19.11%),  $\alpha$ -phellandrene (3.20%) and  $\Delta^3$ -carene (2.75%) were the main compounds of Jebel Mansour EO. Concerning EOs from Siliana and Tabarka, they were characterized by oxygenated monoterpenes with amounts of 39.88% and 43.44% of the total oil, respectively. This class was followed by the sesquiterpene and monoterpene hydrocarbons for the two sites with, respectively, 43.44% and 4.71% from Tabarka and 39.88% and 8.03% from Siliana. Our results are in accordance with those of Zrira et al. (2003) who described variability in Moroccan EO of different regions. Among the 45 constituents identified, the major oil compounds for the region of Oulmes were  $\alpha$ -pinene,  $\beta$ -myrcene (10.2–11.5%) and limonene (6.8–9.8%), whereas terpinene-4-ol (32.7–43.8%),  $\alpha$ -pinene (7.1–13.5%) and bornyl acetate (6.8–10.3%) were the main constituents of Chaouen region.

However, Siliana chemotype presented an EO rich in terpinene-4-ol (23.32%),  $\beta$ -caryophyllene (22.62%) and  $\alpha$ -terpineol (7.22%). The Tabarka's chemotype was mainly composed of  $\beta$ -caryophyllene (38.33%) and  $\alpha$ -terpineol (9.79%). Previous studies reported the variability of *P. lentiscus* L. EO composition from different areas. In Mehdiya samples,

terpinene-4-ol (14.5–19.3%), caryophyllene oxide (6.5–10.3%) and limonene (6.7–10.3%) were the major constituents (Zrira et al. 2003). Previous investigation by Djenane et al. (2011) showed that *P. lentiscus* EO was characterized by a high percentage of  $\beta$ -myrcene (15.18%) and 1.8-cineole (15.02%), followed by terpinene-4-ol (6.41%),  $\alpha$ -pinene (5.54%) and  $\beta$ -pinene (5.10%).

Ben Douissa et al. (2005) identified only 27 compounds, representing 58% of total oil constituents where  $\alpha$ -pinene (17%),  $\gamma$ -terpinene (9%) and terpinene-4-ol (12%) were the main compounds in Tunisian *P. lentiscus* L. EO from Zagouhan region. These results were different when compared to those obtained in our study where 40 compounds were identified. Furthermore, in our research, the presence of limonene in Tunisian *P. lentiscus* L. EO was reported for the first time only in the regions of Jebel Mansour and Siliana. The one from Greece presented qualitative and quantitative differences compared to Tunisian oil samples. Chryssavgi et al. (2008) identified 57 constituents where  $\alpha$ -pinene (9.4–24.9%) and limonene (9.01–17.8%) constituted the major compounds.

A study of 105 samples of EO of *P. lentiscus* L. individual plants from Corsica showed that myrcene, limonene, terpinene-4-ol,  $\alpha$ -pinene,  $\beta$ -pinene,  $\alpha$ -phellandrene, sabinene, *p*-cymene and  $\gamma$ -terpinene were the main constituents (Castola et al. 2000). Spanish EO was characterized by the predominance of the sesquiterpene hydrocarbon fraction (Fernandez et al. 2000). Furthermore,  $\beta$ -caryophyllene (13.1%),  $\gamma$ -cadinene (8.1%) and germacrene-D (6.8%) were the major compounds. These results were different when compared to those reported on Tunisian oil samples where  $\gamma$ -cadinene and germacrene-D were the minor compounds. However, *P. lentiscus* L. EOs from Italy present  $\beta$ -pinene (18.71%),  $\beta$ -phellandrene (12.83%) and  $\beta$ -caryophyllene (13.22%) as the dominating compounds (Congiu et al. 2002). The chemical variability could be due to many factors such as geographic areas, individual chemotypes, harvest time and plant part distilled (Perry et al. 1999). Cluster analysis (Figure 2) was carried out in order to determine the relationship between the different studied regions on the basis of their EO composition. The dendrogram presented in Figure 2 confirms the results consigned in Table II. The application of cluster analysis test to the results obtained from the EO analysis from different Tunisian sites allowed to establish three chemotypes: the first one observed in Oued El Bir and Siliana containing terpinene-4-ol (41.24%) and  $\alpha$ -terpineol as the major compounds. In the second chemotype existing in Jebel Mansour,  $\alpha$ -pinene (9.48%) and limonene (19.11%) were the main compounds. Finally, Tabarka EO constituted

Table II. Composition (% w/w) of leaf EOs from four Tunisian *P. lentiscus* L. localities.

Compound	RI	RI	Identification <sup>c</sup>	Region of collection			
				Oued El Bir	Jebel Mansour	Siliana	Tabarka
E-2-hexenal	850	1219	GC-MS	–	1.29 ± 0.67 <sup>a</sup>	–	–
Z-3-hexenol	855	1370	GC-MS	0.16 ± 0.15 <sup>b</sup>	0.85 ± 0.57 <sup>a</sup>	1.68 ± 0.93 <sup>ab</sup>	0.98 ± 0.11 <sup>a</sup>
E-2-hexenol	856	1356	GC-MS, Co GC	0.09 ± 0.09 <sup>a</sup>	0.54 ± 0.47 <sup>a</sup>	0.36 ± 0.40 <sup>a</sup>	0.15 ± 0.10 <sup>a</sup>
Hexanol	865	1354	GC-MS	0.90 ± 0.55 <sup>a</sup>	0.23 ± 0.12 <sup>b</sup>	–	–
Tricyclene	924	1014	GC-MS, Co GC	1.38 ± 1.27 <sup>a</sup>	1.75 ± 1.40 <sup>a</sup>	2.09 ± 2.09 <sup>a</sup>	0.53 ± 0.03 <sup>a</sup>
α-Thujene	928	1035	GC-MS, Co GC	0.23 ± 0.22 <sup>a</sup>	1.08 ± 0.52 <sup>a</sup>	0.58 ± 0.65 <sup>a</sup>	1.90 ± 2.15 <sup>a</sup>
α-Pinene	939	1032	GC-MS, Co GC	0.60 ± 0.39 <sup>b</sup>	9.48 ± 9.29 <sup>a</sup>	0.67 ± 0.69 <sup>b</sup>	1.44 ± 0.09 <sup>b</sup>
Camphor	954	1076	GC-MS, Co GC	0.88 ± 0.10 <sup>a</sup>	0.17 ± 0.15 <sup>b</sup>	0.72 ± 0.11 <sup>a</sup>	0.43 ± 0.49 <sup>ab</sup>
Camphene	954	1076	GC-MS	0.10 ± 0.09 <sup>a</sup>	1.00 ± 0.93 <sup>a</sup>	1.33 ± 1.29 <sup>a</sup>	0.18 ± 0.05 <sup>a</sup>
Sabinene	975	1132	GC-MS	0.07 ± 0.06 <sup>b</sup>	1.42 ± 1.16 <sup>a</sup>	0.32 ± 0.36 <sup>b</sup>	0.16 ± 0.18 <sup>b</sup>
β-Pinene	980	1118	GC-MS	0.24 ± 0.22 <sup>a</sup>	3.27 ± 1.64 <sup>a</sup>	1.84 ± 2.08 <sup>a</sup>	0.40 ± 0.07 <sup>a</sup>
Myrcene	991	1174	GC-MS	–	1.12 ± 1.27 <sup>a</sup>	0.35 ± 0.39 <sup>a</sup>	–
α-Phellandrene	1006	1176	GC-MS	–	3.20 ± 0.77 <sup>a</sup>	0.45 ± 0.50 <sup>b</sup>	–
Δ <sup>3</sup> -Carene	1011	1059	GC-MS	–	2.75 ± 2.12 <sup>a</sup>	0.38 ± 0.33 <sup>b</sup>	0.33 ± 0.37 <sup>a</sup>
<i>p</i> -Cymene	1026	1280	GC-MS	–	0.06 ± 0.07 <sup>a</sup>	–	0.14 ± 0.16 <sup>a</sup>
<b>Limonene</b>	1030	1203	GC-MS	–	<b>19.11 ± 21.63<sup>a</sup></b>	0.34 ± 0.16 <sup>a</sup>	–
1-8-Cineole	1033	1213	GC-MS	–	2.04 ± 2.30 <sup>a</sup>	0.80 ± 0.90 <sup>a</sup>	0.80 ± 0.19 <sup>a</sup>
( <i>E</i> )-β-Ocimene	1050	1266	GC-MS	–	0.29 ± 0.32 <sup>a</sup>	0.13 ± 0.15 <sup>a</sup>	–
γ-Terpinene	1053	1243	GC-MS	–	0.16 ± 0.18 <sup>a</sup>	0.35 ± 0.39 <sup>a</sup>	0.38 ± 0.01 <sup>a</sup>
<i>Cis</i> -linalool oxide	1074	1450	GC-MS	0.30 ± 0.14 <sup>a</sup>	0.45 ± 0.50 <sup>a</sup>	0.07 ± 0.08 <sup>a</sup>	0.30 ± 0.03 <sup>a</sup>
<i>Trans</i> -linalool oxide	1088	1475	GC-MS	0.50 ± 0.23 <sup>a</sup>	0.19 ± 0.22 <sup>a</sup>	0.36 ± 0.21 <sup>a</sup>	0.13 ± 0.15 <sup>a</sup>
Terpinolene	1092	1290	GC-MS	–	0.37 ± 0.42 <sup>a</sup>	–	–
Linalool	1098	1553	GC-MS	0.41 ± 0.17 <sup>ab</sup>	0.53 ± 0.06 <sup>a</sup>	0.58 ± 0.06 <sup>a</sup>	0.17 ± 0.19 <sup>b</sup>
Borneol	1165	1702	GC-MS	0.39 ± 0.10 <sup>b</sup>	0.88 ± 0.46 <sup>a</sup>	0.60 ± 0.20 <sup>ab</sup>	0.16 ± 0.18 <sup>b</sup>
<b>Terpinene-4-ol</b>	1178	1611	GC-MS	<b>41.24 ± 10.97<sup>a</sup></b>	1.53 ± 0.27 <sup>b</sup>	<b>23.32 ± 18.41<sup>a</sup></b>	0.80 ± 0.91 <sup>b</sup>
<b>α-Terpineol</b>	1189	1709	GC-MS	7.31 ± 1.94 <sup>a</sup>	2.13 ± 1.84 <sup>b</sup>	<b>7.12 ± 1.65<sup>a</sup></b>	9.79 ± 9.32 <sup>b</sup>
Geraniol	1255	1857	GC-MS	0.36 ± 0.25 <sup>ab</sup>	0.07 ± 0.08 <sup>b</sup>	0.28 ± 0.06 <sup>a</sup>	0.11 ± 0.01 <sup>b</sup>
Bornyl acetate	1295	1597	GC-MS	1.22 ± 0.41 <sup>a</sup>	0.13 ± 0.01 <sup>b</sup>	0.84 ± 0.18 <sup>ab</sup>	–
Tridecane	1300	1300	GC-MS	–	0.09 ± 0.10 <sup>a</sup>	–	–
Linalyl-propionate	1325	1597	GC-MS	1.75 ± 0.10 <sup>b</sup>	1.53 ± 0.27 <sup>b</sup>	1.70 ± 1.91 <sup>a</sup>	0.26 ± 0.30 <sup>b</sup>
α-Terpenyl acetate	1344	1706	GC-MS	0.73 ± 0.21 <sup>b</sup>	2.19 ± 1.45 <sup>a</sup>	1.19 ± 0.002 <sup>ab</sup>	–
α-Cubebene	1351	1472	GC-MS	tr	tr	tr	tr
Copaene	1372	1490	GC-MS	0.46 ± 0.11 <sup>b</sup>	0.29 ± 0.09 <sup>b</sup>	1.48 ± 0.08 <sup>a</sup>	0.60 ± 0.68 <sup>b</sup>
β-Elemene	1391	1600	GC-MS	0.83 ± 0.03 <sup>a</sup>	0.28 ± 0.10 <sup>b</sup>	–	–
<b>β-Caryophyllene</b>	1434	1594	GC-MS, Co GC	0.91 ± 0.37 <sup>b</sup>	1.11 ± 0.92 <sup>b</sup>	<b>22.62 ± 24.15<sup>b</sup></b>	38.33 ± 42.51 <sup>a</sup>
α-Humulene	1454	1687	GC-MS	0.54 ± 0.04 <sup>a</sup>	–	1.14 ± 0.03 <sup>a</sup>	–
Allo-aromadendrene	1474	1661	GC-MS	0.44 ± 0.04 <sup>a</sup>	–	0.27 ± 0.02 <sup>b</sup>	–
Δ-Muurolene	476	1675	GC-MS	0.37 ± 0.13 <sup>b</sup>	0.29 ± 0.14 <sup>b</sup>	0.97 ± 0.50 <sup>a</sup>	–
Germacrene-D	1480	1726	GC-MS, Co GC	0.30 ± 0.19 <sup>b</sup>	0.38 ± 0.25 <sup>b</sup>	0.85 ± 0.02 <sup>a</sup>	–
Nonadecanone	1900	1900	GC-MS	0.08 ± 0.07 <sup>c</sup>	0.36 ± 0.26 <sup>a</sup>	–	–
<i>Grouped compound</i>							
Monoterpene hydrocarbons				2.64 <sup>c</sup>	<b>45.12<sup>a</sup></b>	8.03 <sup>b</sup>	4.71 <sup>ab</sup>
Oxygenated monoterpenes				<b>55.14<sup>a</sup></b>	19.38 <sup>d</sup>	<b>39.88<sup>c</sup></b>	<b>43.44<sup>b</sup></b>
Aliphatic hydrocarbons				0.08 <sup>b</sup>	0.45 <sup>a</sup>	–	–
Alcohols				1.98 <sup>a</sup>	1.62 <sup>ab</sup>	2.05 <sup>a</sup>	1.02 <sup>b</sup>
Sesquiterpenes				3.87 <sup>c</sup>	2.71 <sup>c</sup>	<b>25.94<sup>b</sup></b>	<b>31.43<sup>a</sup></b>
Others				9.82 <sup>b</sup>	4.14 <sup>c</sup>	8.47 <sup>b</sup>	10.58 <sup>a</sup>
Total				73.53	73.42	84.37	91.18

Notes: Components are listed in order of elution in polar column (HP-Innowax); “–” = compound not detected; RI: RIs calculated using, respectively, an apolar column (HP-5) and polar column (HP-Innowax). Means of three replicates ± SD. Values with different superscripts (a–d) are significantly different at  $P < 0.05$ . Bold values have been used for showing the main representative compounds.

the third chemotype with β-caryophyllene (38.33%) and α-terpineol (9.79%) as major compounds.

#### Antioxidant activity

Changes observed in EO composition from the four Tunisian regions led us to investigate their

antioxidant activities. Since a single method gives only a reductive suggestion on the antioxidant capacity of any sample (Gianni et al. 2005), antioxidant activities of *P. lentiscus* EOs from four Tunisian localities were tested by different methods based on DPPH radical scavenging, β-carotene–linoleic acid bleaching, metal chelating activity and



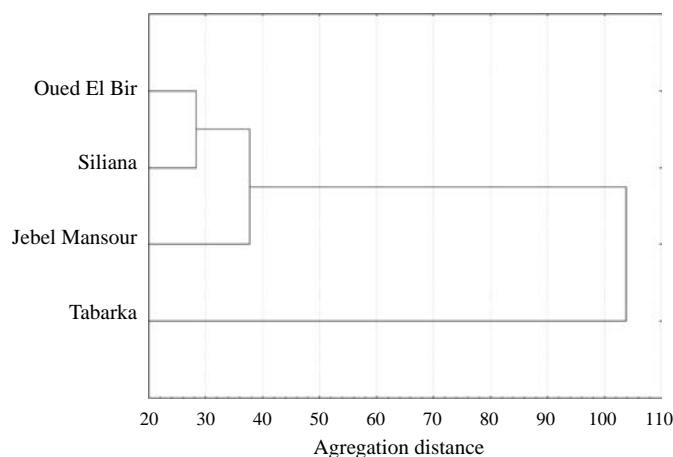


Figure 2. Cluster analysis from Tunisian populations of *P. lentiscus* based on their EO composition.

reducing power assays. Lower  $IC_{50}$  value indicated higher antioxidant activity. All the EO samples showed antioxidant activity but significantly statistical differences were only observed when considering provenance criteria.

Table III gathers the statistical analysis of  $IC_{50}$  values obtained from leaf EO at the four locations. It is a direct linear relationship between the EO concentration in the DPPH solution and the inhibition percentages with a correlation factor  $R^2 \geq 0.923$ . The results of DPPH assay gives an  $IC_{50}$  range value of 60–110.8  $\mu\text{g}/\text{mL}$  for all the samples studied. *P. lentiscus* EO from Tabarka showed the higher antioxidant activity with an  $IC_{50}$  of 60  $\mu\text{g}/\text{mL}$ . According to the mean value of each location, it seems that Jebel Mansour and Siliana have the same value  $IC_{50} = 100\text{--}100.8 \mu\text{g}/\text{mL}$ . Moreover, Oued El Bir location indicated an  $IC_{50} = 98 \mu\text{g}/\text{mL}$ . After comparison with BHT and BHA, obtained results indicated that leaf EOs from Tunisian *P. lentiscus* exhibited a weak antioxidant activity in terms of

scavenging DPPH free-radical assay. The  $IC_{50}$  variations could be explained by the chemical composition variability of these oil samples from different origins. Our results are in accordance with those of Gourine et al. (2010) who mentioned that leaf EOs from Algerian *P. atlantica* had a weak antioxidant activity in terms of scavenging DPPH free radicals with an  $IC_{50} = 17.22 \text{ mg}/\text{mL}$  for Laghouat region and  $IC_{50} = 18.95 \text{ mg}/\text{mL}$  for Hassi R'mel region. However, radical scavenging capacity is directly related to the hydrogen-atom-donating ability of a compound (Molyneux 2004; Kumaran & Karunakaran 2007). So, trying to correlate the observed activity with the chemical composition of the EOs, it is well known that *P. lentiscus* EO was characterized by the predominance of monoterpenes ( $\alpha$ -pinene,  $\beta$ -pinene and limonene). As reported elsewhere, the components mentioned above were all tested individually and none exhibited strong antioxidative activity with the same procedure like DPPH assay (Burits & Bucar

Table III. Antioxidant activities of leaf EOs from four Tunisian *P. lentiscus* L. localities.

	DPPH ( $IC_{50}$ , $\mu\text{g}/\text{ml}$ )	$\beta$ -Carotene bleaching ( $IC_{50}$ , $\mu\text{g}/\text{ml}$ )	Chelating ability ( $IC_{50}$ , $\text{mg}/\text{ml}$ )	Reducing power ( $EC_{50}$ , $\mu\text{g}/\text{ml}$ )
<i>EO</i>				
Site 1: Oued El Bir	98 $\pm$ 0.004 <sup>c</sup>	110 $\pm$ 0.036 <sup>a</sup>	80.8 $\pm$ 0.032b <sup>c</sup>	250 $\pm$ 0.028 <sup>a</sup>
Site 2: Jebel Mansour	100 $\pm$ 0.002 <sup>b</sup>	100 $\pm$ 0.057 <sup>b</sup>	116 $\pm$ 0.076 <sup>a</sup>	100 $\pm$ 0.039 <sup>b</sup>
Site 3: Siliana	110.8 $\pm$ 0.004 <sup>a</sup>	100 $\pm$ 0.041 <sup>b</sup>	88 $\pm$ 0.178 <sup>c</sup>	250 $\pm$ 0.041 <sup>a</sup>
Site 4: Tabarka	60 $\pm$ 0.01 <sup>d</sup>	108 $\pm$ 0.033 <sup>a</sup>	104 $\pm$ 0.028 <sup>b</sup>	250 $\pm$ 0.043 <sup>a</sup>
<i>Synthetic antioxidant</i>				
BHT	25 $\pm$ 0.20 <sup>c</sup>	70 $\pm$ 0.57 <sup>c</sup>	–	–
EDTA	–	–	1.03 $\pm$ 0.12 <sup>d</sup>	–
Ascorbic acid	–	–	–	40 $\pm$ 0.13 <sup>c</sup>
BHA	–	43 $\pm$ 0.15 <sup>d</sup>	–	–

Notes:  $EC_{50}$  value: the effective concentration at which the antioxidant activity was 50%; the absorbance was 0.5 for reducing power; DPPH radicals were scavenged by 50% and ferrous ions were chelated by 50%, respectively. The  $EC_{50}$  value was obtained by interpolation from linear regression analysis. Each value is expressed as mean  $\pm$  SD ( $n = 4$ ). Means with different capital letter within a row are significantly different ( $P < 0.05$ ).

2000; Kelen & Tepe 2008). Generally, EOs rich in thymol and carvacrol exhibited a strong antioxidant activity like *Thymus capitatus* (Bounatirou et al. 2007).

Bleaching  $\beta$ -carotene with the linoleic acid system as antioxidant activity of *P. lentiscus* EOs from four localities was tested. Table III shows that all samples exhibited lower antioxidant activity than BHA and BHT with  $IC_{50}$  values of 110, 108, 100 and 100  $\mu\text{g/mL}$  in Oued El Bir, Jebel Mansour, Siliana and Tabarka, respectively. As for antiradical scavenging activity, *P. lentiscus* EO samples were characterized by a low ability to prevent the bleaching of  $\beta$ -carotene. Actually, reducing power is a very important aspect for the estimation of the antioxidant activity (Meir et al. 1995). The presence of antioxidants in the solution causes the reduction of the  $\text{Fe}^{3+}$ –ferricyanide complex to the ferrous form. As shown in Table III, all *P. lentiscus* EO samples exhibited a very low reducing activity which is lower than that of ascorbic acid. The reductive potential reached an  $IC_{50}$  value of 100  $\mu\text{g/mL}$  in Jebel Mansour and 250  $\mu\text{g/mL}$  in Oued El Bir, Siliana and Tabarka. These results are not in accordance with those obtained by Gourine et al. (2010) who states that *P. atlantica* EOs from four different locations in Algeria have a higher antioxidant capacity relative to the antioxidant of reference ascorbic acid. Indeed, the oils are nearly 3–11 times more active than ascorbic acid. It seem to act as an electron donator, which reacts with free radicals converting them with more stable products and thereby terminates radical chain reaction as described by Shimada et al. (1992). Moreover, the reductive potential may be due to the di- and monohydroxyl substitutions in the aromatic ring (Shimada et al. 1992). Furthermore, Chryssavgi et al. (2008) indicated that *P. lentiscus* L. methanolic extracts exhibit a strong reducing power with an  $IC_{50}$  range value of 84.6–131.4  $\text{mmol Fe}^{2+}/\text{L}$  plant extract.

The ability to chelate transition metals can be considered as an important antioxidant mode of action. According to the results presented in Table III, we can note that *P. lentiscus* EO from different areas showed no metal chelating activity with  $IC_{50}$  ranging from 80.8 to 116  $\text{mg/mL}$ . In this essay, EDTA exhibited an  $IC_{50}$  of 1.03  $\text{mg/mL}$ . These results are in accordance with those obtained by Aidi Wannes et al. (2010) who indicated that the EO of leaf and stem showed no metal chelating activity, whereas flower EO presented a very low ability to chelated ferrous irons. Moreover, Bounatirou et al. (2007) reported that both *Thymus capitatus* EOs and BHA and BHT showed no metal chelating activity. These authors revealed that BHA and carvacrol, the main component of EO, are monohydroxylated compounds which are not able to form complex with  $\text{Fe}^{2+}$ . However, it has been noted that dihydroxylated components are able to chelate

transition metals in the case of clove EO (Jirovetz et al. 2006). Thus, the chelating effect was stronger than the positive control EDTA. In conclusion, *P. lentiscus* EOs from different areas were rich in monohydroxylated compounds justifying the obtained results.

## Conclusion

During the flowering stage, *P. lentiscus* L. EOs were characterized by their relatively low yields. Three chemotypes of *P. lentiscus* EO were identified for the first time in Tunisia. Many variations in the oil chemical composition between the four Tunisian regions were observed. Furthermore, the presence of limonene in Tunisian *P. lentiscus* L. EO was reported for the first time only in the regions of Jebel Mansour and Siliana, representing a new chemotype. So, these EOs presented many bioactive compounds that could have numerous applications in food, pharmaceutical, cosmetic and perfume industries. The DPPH radical scavenging,  $\beta$ -carotene–linoleic acid bleaching, reducing power and metal chelating activity assays revealed low antioxidant activities of *P. lentiscus* EO leaves.

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