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Seasonal Variation of Chemical Composition and Antioxidant Activity of Essential Oil from *Pistacia atlantica* Desf. Leaves

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Abstract The composition and antioxidant activities of *Pistacia atlantica* Desf. essential oil were investigated. Qualitative and quantitative differences in compositions and in antioxidant activities of male and female leaf essential oils were observed during the season. The essential oils obtained by hydrodistillation were analysed by GC and GC–MS. The oils were rich in monoterpenes hydrocarbons and oxygenated sesquiterpenes. The main components of male essential oil were α-pinene/α-thujene, spathulenol and bicyclogermacrene. The major component of female essential oil was δ-3-carene. The seasonal variation showed that most of the main components of the oils reached theirs highest values in September. The antioxidant activity of the oil was investigated in vitro using two assays: DPPH· (2,2-di-phenyl-1-picrylhydrazyl) free radical-scavenging and FRAP (Ferric Reducing Antioxidant Power). The highest antioxidant capacity to scavenge free DPPH radicals was reached in the month of June for male oils and during the months of September–October for the female oils. The high reducing power for male oil was observed during the month of June and for the female oil it was in August. The female oil was more active than the male oil. The antioxidant capacity of the female oil was almost ten times higher than Ascorbic acid in the FRAP assay.

Keywords *Pistacia atlantica* · Essential oil · Antioxidant activity · DPPH · FRAP

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Introduction

The genus Pistacia (Anacardiaceae) is widely distributed in the Mediterranean area [1]. *Pistacia atlantica* Desf. is a tree located in North Africa, which can reach over 15 m in height and grows in arid and semi-arid areas [2]. *Pistacia Atlantica* is valued because it is the source of mastic gum, an exudate which strengthens gums, deodorises breath, fights coughs, chills and stomach diseases [3]. Moreover, the galls of *Pistacia atlantica* are used as an embalming agent by rural inhabitants. They are also known in Arabic as "afse" and are edible and sold in markets.

This is the first report that aims to investigate the change in the chemical composition of essential oil from leaves of *Pistacia atlantica* (male and female) collected over several months during the growing season. A second objective was to assess the DPPH radical and FRAP activity of the essential oils.

Materials and Methods

Chemicals

Ethanol, sodium acetate trihydrate, TPTZ (2,4,6-tripyridyl-*s*-triazine), hydrochloric acid HCl, 2,2-diphenyl-1-picryl-

hydrazyl (DPPH) and mixtures of homologous *n*-alkanes series C_8–C_{20}, C_{21}–C_{40} were obtained from Sigma-Aldrich (Germany). Ascorbic acid and butylated hydroxyanisole (BHA) were obtained from Fluka (Switzerland). Acetic acid and sodium sulphate were obtained from Prolabo Merk. $Fe_2SO_4.7H_2O$ was obtained from Panreac (Spain).

Plant Material

Fresh leaves, from male and female plants of *Pistacia atlantica* Desf., were collected periodically at the beginning of each month starting in June and ending by October 2007. The plants were obtained from the same wild area and the same trees located 8 km south east of the town of Laghouat, 400 km south of Algiers, in a region which belongs to the Saharian Atlas, having a Mediterranean climate.

Preparation of Samples

An extract of air-dried leaves of *Pistacia atlantica* was prepared by water distillation using a Clevenger apparatus for almost 8 h, which produced 0.09–0.13% v/w. The oil obtained was dried over anhydrous sodium sulphate, filtred and stored at +4 °C until it was tested and analysed.

GC Analysis

A CP-Varian 3800 gas chromatograph was used with a flame ionisation detector (FID), and a UB-Wax fused silica capillary column (60 m × 0.32 mm, 0.25 μm film thickness). The oven temperature was programmed from 50 to 250 °C at a rate of 3 °C/min and held at 250 °C for 10 min. Injector and detector temperatures were set at 250 and 260 °C respectively. Helium was the carrier gas at a flow rate of 1 mL/min. Linear retention indices were calculated with reference to *n*-alkanes (C_8–C_{40}).

GC–MS Analysis

The chemical composition of the *Pistacia atlantica* leaves essential oil was analysed using an AGILENT 6890 GC/CMSD 5973 equipped with a capillary column HP5MS (30 m × 0.25 mm, 0.25 μm film thickness) and a 70-eV EI Quadruapole detector. Helium was the carrier gas, at a flow rate of 1 mL/min. Injector and MS transfer line temperatures were set at 250 and 220 °C, respectively. The column temperature was initially held at 60 °C for 2 min, then gradually increased to 125 °C at a rate of 2 °C/min, held for 2 min, and finally increased to 220 °C at 5 °C/min and again held for 2 min. Diluted samples (1:100 v/v, in ethanol) of 1.0 μL were injected manually using a splitless mode.

The identifications of the components were based on the comparison of their mass spectra with those of Wiley and NIST (National Institute of Standards and Technology) libraries, as well as by comparison of their retention indices with those of the values of a homemade database and by co-injections of some pure authentic samples.

DPPH Assay

The method is based on the reduction of alcoholic DPPH solutions in the presence of a hydrogen donating antioxidant. DPPH solutions show a strong absorption band at 517 nm with a deep violet colour. The absorption vanishes and the resulting decolouration is stoichiometric with respect to degree of reduction. The remaining DPPH, measured after a certain time, corresponds inversely to the radical scavenging activity of the antioxidant [4–8].

Different concentrations of essential oils in ethanol were prepared; each solution (50 μL) was mixed with 1 mL of ethanolic solution containing DPPH radicals with a concentration 100 μM. After 30 min of incubation at room temperature, the absorbance was read against a blank at 517 nm (Shimadzu UV/Vis 1601 apparatus). Inhibition of DPPH free radical in percent (I%), was calculated as follows:

$$I\% = (1 - \frac{A_{\text{sample}}}{A_{\text{blank}}}) \times 100\%$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound.

Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against extract concentration. All tests were carried out at least in triplicate.

FRAP Assay

Determination of ferric reducing antioxidant power FRAP is a simple direct test for measuring antioxidant capacity. This method was initially developed to assay plasma antioxidant capacity, but can be used with plant extracts too. The FRAP assay was performed as described by Benzie et al. [9–11]. The FRAP reagent was prepared by mixing 300 mM of acetate buffer, pH 3.6 [3.1 g sodium acetate trihydrate, plus 16 mL glacial acetic acid made up to 1 L with distilled water]; 10 mM TPTZ (2,4,6-tripyridyl-*s*-triazine) in 40 mM HCl; and 20 mM $Fe_2SO_4.7H_2O$ in the ratio of 10:1:1 to give the working FRAP reagent. The working reagent was freshly prepared as required. Different concentrations of essential oils in ethanol were prepared; each solution (100 μL) was mixed with 2 mL of working FRAP reagent, and the absorbance was read at

Table 1 Seasonal variation of chemical composition of essential oil of leaves (male and female) of *Pistacia atlantica*

No.	Components	GC area (%)[a]										LRI[b]	Identification
		June		July		August		September		October			
		Male	Female	Male	Female	Male	Female	Male	Female	Male	Female		
1	Tricyclene	1.94	tr	1.84	tr	1.49	tr	1.66	tr	1.26	tr	1016	MS, RI
2	**α-Pinene + α-Thujene**	**21.68**	0.90	**21.30**	tr	**20.32**	0.93	**31.00**	1.13	**21.13**	0.95	1028	MS, RI
3	**Camphene**	**6.39**	0.53	**6.24**	tr	**5.30**	0.53	**5.71**	0.59	**4.41**	0.52	1053	MS, RI
4	***β*-Pinene**	**4.47**	tr	**4.11**	tr	**4.53**	tr	**5.69**	0.39	**4.41**	tr	1118	MS, RI
5	Verbenene	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	1129	MS, RI
6	**δ-3-Carene**	tr	**41.13**	0.52	**16.37**	tr	**49.60**	0.31	**56.18**	2.26	**44.95**	1160	MS, RI
7	α-Phellandrene	tr	tr	tr	tr	tr	0.56	tr	0.55	tr	0.52	1174	MS, RI
8	α-Terpinene	tr	1.02	tr	0.90	tr	0.82	tr	0.86	tr	0.53	1189	MS, RI
9	Limonene	0.71	1.55	0.92	0.60	0.79	1.56	0.74	1.85	0.70	1.45	1209	MS, RI, AS
10	*β*-Phellandrene	tr	0.54	tr	0.41	tr	0.74	tr	0.84	tr	0.60	1219	MS, RI
11	*trans*-2-hexenal	tr	tr	tr	0.43	tr	0.34	tr	tr	tr	tr	1235	MS, RI
12	γ-Terpinene	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	1255	MS, RI
13	*p*-Cymene	tr	0.61	tr	tr	tr	0.45	0.39	0.45	tr	0.54	1282	MS, RI
14	α-Terpinolene	tr	0.87	0.47	tr	tr	1.57	tr	1.76	0.30	tr	1293	MS, RI
15	6-Methyl-5-hepten-2-one	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	1336	MS, RI
16	(*E*)-3-Hexen-1,ol	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	1351	MS, RI
17	*trans*-2-Hexenol	tr	tr	tr	0.42	tr	tr	tr	tr	tr	tr	1361	MS, RI
18	(*Z*)-3-Hexen-1,ol	0.46	tr	tr	1.50	tr	tr	tr	tr	tr	tr	1396	MS, RI
19	2-Hexen-1-ol	tr	0.89	0.37	tr	tr	0.35	tr	0.62	tr	0.45	1406	MS, RI
20	*β*-Thujone	tr	1.32	0.43	1.68	tr	1.07	tr	0.69	tr	1.01	1456	MS, RI
21	Bornylene	tr	0.82	tr	tr	tr	0.57	0.73	0.56	1.10	0.83	1540	MS, RI
22	Linalool	tr	0.63	tr	tr	tr	1.28	tr	1.29	tr	1.28	1557	MS, RI, AS
23	Bornyl acetate	2.46	tr	4.08	1.11	1.51	tr	1.33	0.42	1.16	tr	1595	MS, RI
24	Camphene hydrate	tr	tr	tr	tr	tr	tr	0.27	tr	0.30	tr	1604	MS, RI
25	Terpinen-4-ol	0.67	0.66	0.61	tr	0.61	0.35	0.79	0.50	0.92	tr	1617	MS, RI, AS
26	Aromadendrene	1.68	tr	0.77	tr	1.29	tr	1.17	tr	1.56	tr	1658	MS, RI, AS
27	*allo*-Aromadendrene	1.68	0.87	0.97	0.60	1.16	tr	0.79	0.29	1.42	tr	1657	MS, RI, AS
28	(*E*)-Pinocarveol	1.95	tr	1.22	0.86	tr	tr	1.07	0.46	0.36	tr	1671	MS, RI
29	Ledene	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	1678	MS, RI
30	Linalyl propionate	3.15	3.40	3.32	3.15	1.54	2.50	1.04	1.54	1.04	2.46	1700	MS, RI
31	α-Terpineol	2.76	0.93	2.43	1.50	**5.12**	0.65	4.71	1.30	6.53	1.71	1712	MS, RI, AS
32	**α-Terpenyl acetate**	0.41	**5.31**	0.44	3.60	tr	**5.22**	tr	**6.94**	0.32	**7.00**	1722	MS, RI
33	**Bicyclogermacrene**	3.64	4.37	1.64	3.92	**5.28**	4.36	**7.39**	2.52	**9.89**	4.17	1750	MS, RI
34	Germacrene B	0.56	1.03	0.42	0.59	0.94	0.76	0.87	0.84	1.24	0.98	1846	MS, RI
35	Geranyl acetone	tr	0.70	tr	0.61	0.41	0.44	0.30	0.29	tr	0.76	1869	MS, RI
37	Palustrol	0.48	0.63	tr	tr	tr	tr	0.54	tr	tr	tr	1960	MS, RI
38	Epiglobulol	0.40	0.80	tr	1.32	0.52	0.39	0.43	tr	0.52	tr	2028	MS, RI
39	**Ledol**	1.59	0.76	0.81	0.76	**4.82**	0.59	1.97	0.34	2.32	tr	2050	MS, RI, AS
40	**Globulol**	**3.80**	1.27	**2.13**	1.55	**1.74**	1.19	**4.31**	0.97	**5.49**	1.36	2070	MS, RI, AS
41	Viridiflorol	1.36	tr	0.54	tr	tr	tr	1.52	tr	2.38	tr	2100	MS, RI, AS
42	**Spathulenol**	**12.92**	3.22	**16.71**	2.66	**15.66**	4.13	**12.05**	4.27	**13.41**	**5.00**	2143	MS, RI
43	γ-Eudesmol	1.66	0.53	3.00	0.64	1.51	1.50	1.58	0.88	0.74	1.63	2186	MS, RI
44	**Isospathulenol**	2.60	4.16	4.14	**6.18**	3.06	2.73	2.20	1.78	2.13	**3.57**	2245	MS, RI
45	Phytol	tr	tr	0.72	1.41	0.73	tr	tr	tr	tr	tr	2623	MS, RI, AS
46	Myristic acid	tr	0.68	0.60	tr	2.58	1.84	0.66	tr	tr	tr	2663	MS, RI
47	**Palmitic acid**	1.41	1.25	**4.52**	**11.33**	2.27	0.65	0.82	tr	0.82	0.74	2920	MS, RI

Table 1 continued

No.	Components	GC area (%)[a]										LRI[b]	Identification
		June		July		August		September		October			
		Male	Female	Male	Female	Male	Female	Male	Female	Male	Female		
	Total identified	80.83	81.38	85.27	64.10	83.18	87.67	92.04	91.10	88.12	83.01		
	Monoterpenes hydrocarbons	35.19	47.97	35.4	18.28	32.43	57.33	46.23	65.16	35.57	50.89		
	Oxygenated monoterpenes	8.53	6.94	8.01	7.19	7.27	5.85	7.88	5.78	9.15	6.46		
	Sesquiterpenes hydrocarbons	7.56	6.27	3.8	5.11	8.67	5.12	10.22	3.65	14.11	5.15		
	Oxygenated sesquiterpenes	24.81	11.37	27.33	13.11	27.31	10.53	24.6	8.24	26.99	11.56		
	Fatty acids	1.41	1.93	5.12	11.33	4.85	2.49	1.48	tr[c]	0.82	0.74		
	Essential oil yield % (v/w)	0.13	0.12	0.11	0.09	0.11	0.13	0.12	0.11	0.13	0.12		

tr trace (<0.1%) *RI* retention indices, *MS* mass spectroscopy, *AS* relative to retention indices of pure authentic samples

[a] Percentages obtained by FID peak-area normalisation

[b] Linear retention indices relative to C_8–C_{40} obtained on a UB-Wax column

[c] Sum of all traces

593 nm (Shimadzu UV/Vis 1601 apparatus), the absorption reading was performed at room temperature for a time period of 30 min, against a blank (working FRAP reagent previously prepared without the oil extract). Data were expressed relative to values obtained with Ascorbic acid from calibration curves and then expressed as Ascorbic acid equivalents (AEAC). All the tests were carried out at least in triplicate.

Statistical Analysis

For the antioxidant activities, each result is reported as the mean ± SD of at least three independent replicates. Statistical analysis of data was carried out by computer using MS-Excel. Strictly linear calibration curves were obtained for all of the methods using different calibration standards. Multiple comparison tests were used to analyse data. *P* values less than 0.05 were considered significant.

Results and Discussion

Extract yields of 0.09–0.13% (v/w) were obtained from air-dried leaves (male and female) of *Pistacia atlantica* Desf. The extracts obtained were pale-yellow to light-brown oils with an aromatic-spicy odour. The average extract yields of (0.12 ± 0.01) and (0.11 ± 0.02) v/w were observed for the male and female samples collected from June to October, respectively. This means that the yield could be considered practically unchanged during the season and did not differ for both male and female oils.

The composition of *Pistacia atlantica* essential oil was analysed by GC and GC–MS. Qualitative and quantitative differences in compositions of essential oils were observed. The major difference found was between the composition of male and the female essential oils. Forty-seven compounds were identified (Table 1). The main components of male essential oil were α-pinene/α-thujene (20.3–31.0%), spathulenol (12.5–16.7%) and bicyclogermacrene (1.64–9.89%). This male oil was rich in monoterpenes hydrocarbons (32.4–46.2%) and oxygenated sesquiterpenes (27.6–32.5%). The oxygenated monoterpenes are present in lesser amounts percentage (4.5–6.0%). The fatty acids were present in a range of (0.8–5.1%). The major component of female essential oil was δ-3-carene (16.4–56.2%). This female oil was rich in monoterpenes hydrocarbons (18.3–65.2%) and oxygenated sesquiterpenes (10.9–17.0%). Similar to the male oil, the female oil had a smaller percentage of sesquiterpenes hydrocarbons (3.7–6.3%) but the percentage of fatty acids was more significant (0.7–11.3%).

The composition of the essential oils shows clearly that the female essential oils could be easily and simply differentiated and separated from the male ones based on the composition of δ-3-carene. The male essential oils had a very weak content of δ-3-carene (<2.3%), the female essential oils were characterised by higher percentages of this compound (16.4–56.3%). In the other hand, the male essential oils were characterised by a high percentages of α-pinene/α-thujene (20.3–31.0%) and relatively high contents of camphene (4.4–6.4%) and β-pinene (4.1–5.7%), whereas the female essential oils were characterised by low percentages of the same compounds: α-pinene/α-thujene (<1.1%), camphene (<0.6%) and β-pinene (<0.4%). Moreover, the male essential oils were characterised by higher percentages of bicyclogermacrene (1.6–9.9%), α-terpineol (2.4–6.5%) and globulol (1.7–5.5%) than those of the female essential oils, which contained lower levels of bicyclogermacrene (2.5–4.4%), α-terpineol (0.7–1.7%) and

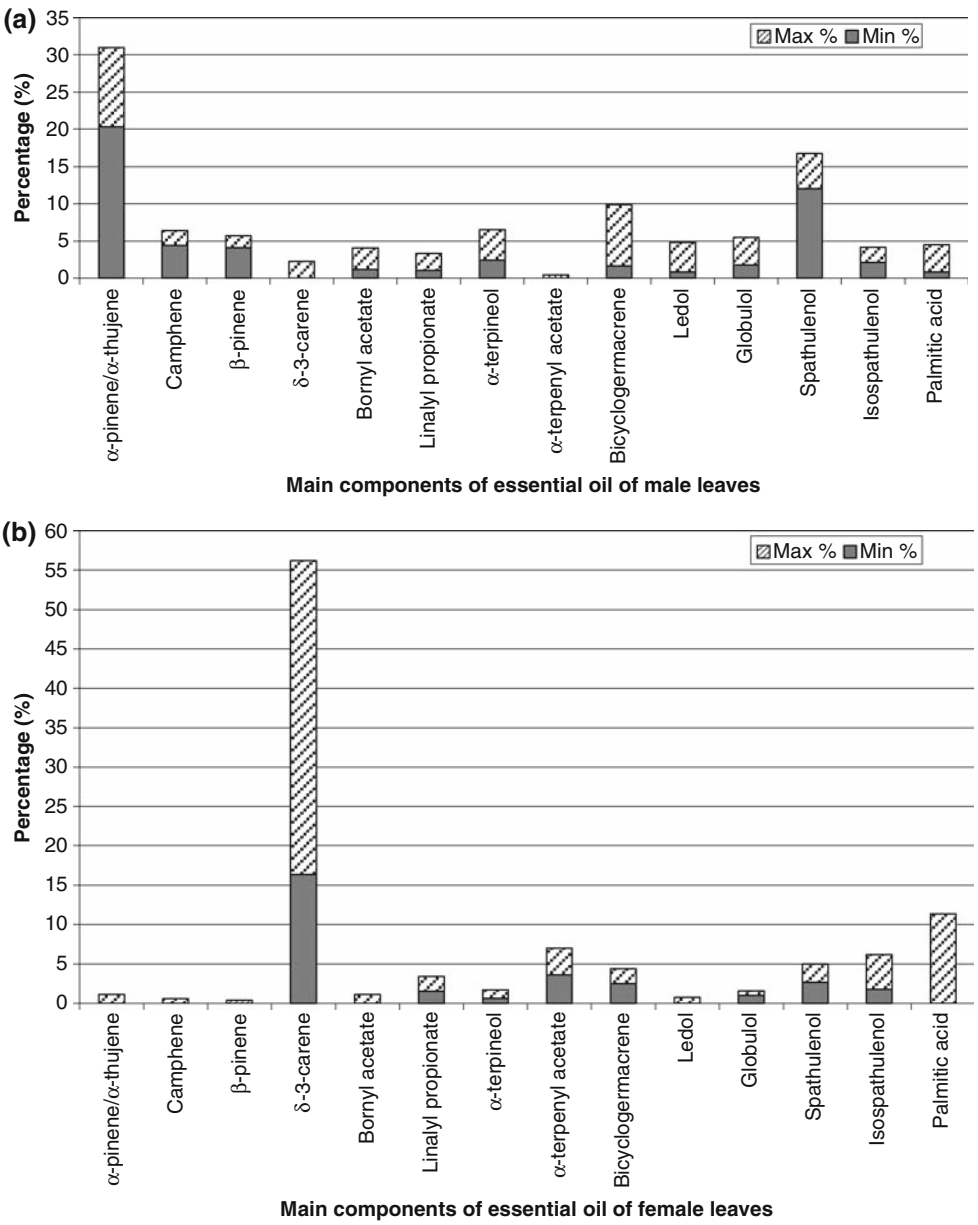

Fig. 1 Maximum and minimum percentages values of mains components of essential oils of *Pistacia atlantica*. **a** male leaves, **b** female leaves

globulol (1.0–1.6%). In addition, the percentage of α-terpenyl acetate was higher in female essential oils (3.6–7.0%) than in male essential oils (<0.4%). The opposite case was recorded for the spathulenol compound, where its percentage was less important in female essential oils (2.7–5.0%) than in male ones (12.5–16.7%). For the rest of the components there is no practically significant influence that could differentiate the male for the female essential oils.

The analysis of the obtained data reveals the existence of two chemotypes which could easily distinguish the male and the female essential oils. The male essential oil was characterised by α-pinene/α-thujene, camphene, *β*-pinene, α-Terpineol, and spathulenol chemotypes, whereas the female oil was characterised by *δ*-3-carene and α-terpenylacetate chemotypes (Fig. 1; Tables 1, 2).

The seasonal variation shows that most of the main components of the oils reached their highest values in September. (Fig. 2; Table 1). The α-pinene + α-thujene contents remained relatively constant in the male essential oils over the growing season except in September where these components increased by almost 10% (Fig. 2a; Table 1). Spathulenol increased slightly in July and August and remained constant for the rest of the season (Fig. 2a). The percentage of bicyclogermacrene seemed to decrease from June to July and then increases linearly from July to October. The camphene with a low percentage remains steady over the season (Fig. 2a). The maximum value of palmitic acid was noted in July for both male and female oils.

The percentage of *δ*-3-carene is steady at the beginning and at the end of the season (June and October) in the

Table 2 Statistical analysis of main identified components of essential oil of leaves (male and female) of *Pistacia atlantica*

Main components	Male				Female			
	Min	Max	Mean	SD	Min	Max	Mean	SD
α-Pinene + α-Thujene	**20.32**	**31.00**	**23.09**	**4.45**	tr	1.13	0.78	0.45
Camphene	**4.41**	**6.39**	**5.61**	**0.80**	tr	0.59	0.43	0.24
***β*-Pinene**	**4.11**	**5.69**	**4.64**	**0.61**	tr	0.39	0.08	0.17
δ-3-Carene	tr	2.26	0.62	0.94	**16.37**	**56.18**	**41.65**	**15.20**
Bornyl acetate	1.16	4.08	2.11	1.21	tr	1.11	0.31	0.48
Linalyl propionate	1.04	3.32	2.02	1.13	1.54	3.40	2.61	0.72
α-Terpineol	**2.43**	**6.53**	**4.31**	**1.71**	0.65	1.71	1.22	0.43
α-Terpenyl acetate	tr	0.44	0.23	0.22	**3.60**	**7.00**	**5.61**	**1.41**
Bicyclogermacrene	**1.64**	**9.89**	**5.57**	**3.21**	2.52	4.37	3.87	0.78
Ledol	0.81	4.82	2.30	1.52	tr	0.76	0.49	0.32
Globulol	**1.74**	**5.49**	**3.49**	**1.56**	0.97	1.55	1.27	0.21
Spathulenol	**12.05**	**16.71**	**14.15**	**1.96**	2.66	5.00	3.86	0.92
Isospathulenol	2.13	4.14	2.83	0.82	**1.78**	**6.18**	**3.68**	**1.66**
Palmitic acid	0.82	4.52	1.97	1.55	tr	**11.33**	**2.79**	**4.79**
Monoterpenes hydrocarbons	32.43	46.23	36.96	5.34	18.28	65.16	47.93	17.84
Oxygenated monoterpenes	7.27	9.15	8.17	0.71	5.78	7.19	6.44	0.63
Sesquiterpenes hydrocarbons	3.80	14.11	8.87	3.77	3.65	6.27	5.06	0.93
Oxygenated sesquiterpenes	24.6	27.33	26.21	1.38	8.24	13.11	10.96	1.78
Fatty acids	0.82	5.12	2.74	2.07	0.74	11.33	4.12	4.60
Essential oil yield % (v/w)	0.11	0.13	0.12	0.01	0.09	0.13	0.11	0.02

tr trace (<0.1%)

female essential oil (Fig. 2b). It decreased in July (16.37%) and reached its maximum value in September (56.18%). The α-terpenyl acetate, isospathulenol and bicyclogermacrene contents did not change significantly over the complete season (Fig. 2b).

Essential oils of leaves of many species of *Pistacia* (*P. lentiscus*, *P. khinjuk*, *P. chinensis*, *P. terebinthus*, *P. palaestina*, *P. Atlantica*) have been investigated. The main constituents of some *Pistacia* species are summarised in Table 3. Comparison of previous studies shows a remarkable variability that seems to depend on plants species and plant organ. In the essential oil obtained from the leaves, some authors found a dominance of monoterpene hydrocarbons [12–17]. In particular, the main constituents of the most studied species, *P. lentiscus*, were α-pinene (Greece and France) [16–18], myrcene (Spain and Sicily) [12, 16], δ-carene (Egypt) [14], and terpinen-4-ol, α-pinene, limonene, and myrcene (Corsica) [17]. According to their intraspecific variability, the essential oil of *P. lentiscus* can be classified into three groups on the basis of their content of terpinen-4-ol, α-pinene, limonene, and myrcene. Although *Pistacia palaestina* is a variety of *Pistacia terebinthus*, the composition of the essential oil of the leaves was very different [19]. In *Pistacia terebinthus*, the monoterpenes terpinen-4-ol, γ-terpinene, limonene, and α-terpinene have been detected as the main compounds, while in *Pistacia palaestina* the main compounds were α-pinene and myrcene [20].

In the literature, there are only a few studies conducted on the essential oil of *Pistacia atlantica* leaves, and among them only one study reported separately the compositions of the male and female oils (Table 3). No study had been carried out to evaluate the components over a growing season. Results obtained by our current investigation and the data from previous reports shows a very large difference between the compositions of the main components. Indeed Barrero et al. [21] reported that the main components are terpinen-4-ol (21.7%) and elemol (20.0%). On the other hand, Tzakou et al. [22] claims that the main components are terpinen-4-ol (17.3%) and *p*-mentha-1(7),8-diene (41.1%) for male essential oil and myrcene (17.8%; 24.8%) and terpinen-4-ol (11.6%; 6.0%) for female oil. It appears that terpinen-4-ol is the major compound in the essential oils of *leaves Pistacia atlantica*. The components of our oil coming from Algeria are quite different from all the previous reports (Morocco and Greece) since male oil was characterised by α-pinene/α-thujene (20.32–31%), spathulenol (12.5–16.71%) and bicyclogermacrene (1.64–9.89%), while the female oil is distinguished only by δ-3-carene (16.37–56.18%). These differences could be due

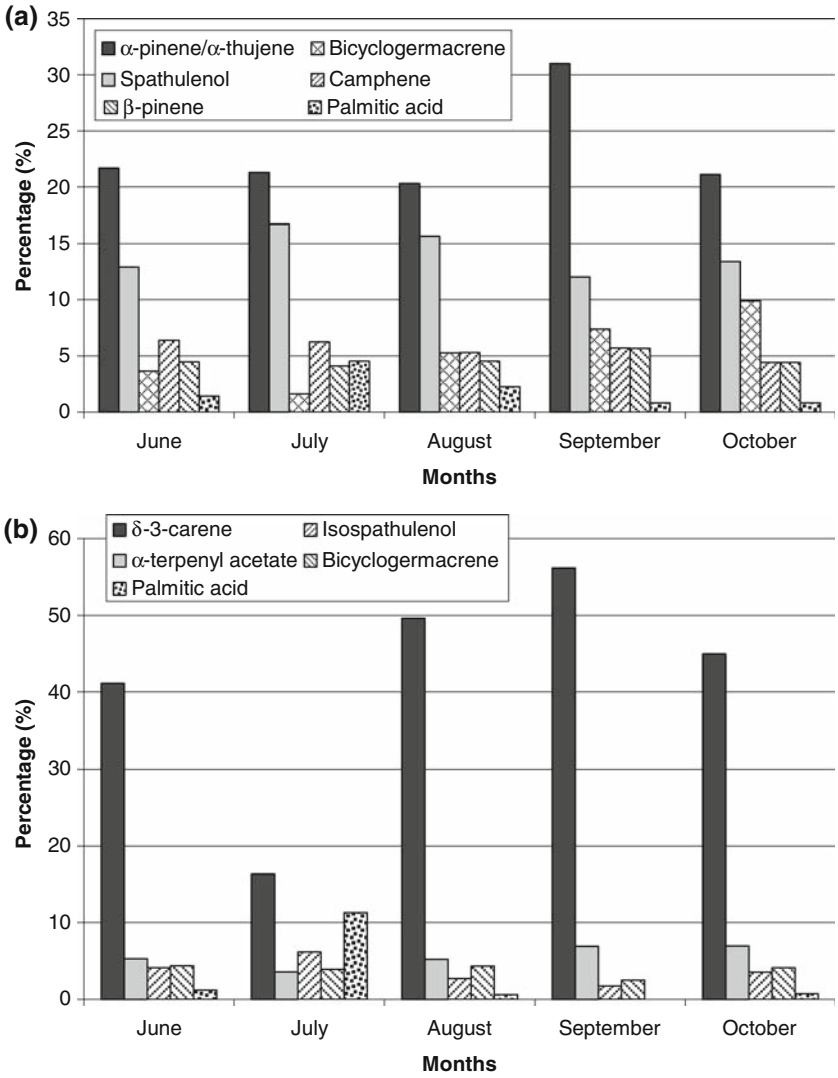

Fig. 2 Seasonal evolution of the mains components of essential oil of *Pistacia atlantica*. **a** male leaves, **b** female leaves

essentially to the difference of the region of growth and maybe also due to the climate and to the tree itself. These differences in the compositions are generally responsible of the several medicinal uses, which varies from one country to another.

The result of the DPPH assay shows that the value of IC_{50} during the season varies between 20.61 and 27.87 mg/mL for male oils and between 8.47 and 17.45 mg/mL for the female oils (Table 4). The highest antioxidant capacity was reached in the month of June for male oils and during the months of September–October for the female oils. The seasonal variations of the IC_{50} for both male and female oils is shown in Fig. 3. It is more convenient to present data as $1/IC_{50}$, known as the antiradical efficiency (AE) or antiradical power (ARP) (Fig. 4), since the lowest value of IC_{50} corresponds to its highest antioxidant capacity. Figure 4 shows that in general, the female oils are more active than the male ones and especially in September and October were almost three times more active. The male oils antioxidant activity decreases very slowly all over the season. The same thing could be said for the female oils only from June to August but in September and October their capacities jumps to almost twice their original values. The antioxidant capacity of the tested oils were weak in comparison with a chosen set of synthetic antioxidants (BHA, BHT and Ascorbic acid), which were used as positive controls (Table 5).

The ferric reducing ability of the plasma (FRAP) assay was used for assessing "antioxidant power" of *Pistacia atlantica* essential oils. The value of AEAC varies between 4.95 and 8.39 mg/mL for male oils and between 9.37 and 11.81 mg/mL for female oils (Table 4). The highest activity for male oil was observed during the month of June and for the female oil it was in August. Figure 5 shows again that the female oils were more active than the male ones. For male oils and during July, September and October the value of AEAC could be considered steady. In August, the value of AEAC increased slightly, but was

Table 3 Main compositions of leaves essential oil of some *Pistacia species* from literature

Plant name	Country of origin	Main constituents of essential oil leaves extract	References
P. Atlantica	Morocco	Terpinen-4-ol (21.7%)	[21]
		Elemol (20.0%)	
	Greece "male tree"	Terpinen-4-ol (17.3%)	[22]
		p-Mentha-1(7),8-diene (41.1%)	
	Greece "female tree"	Myrcene (17.8; 24.8%)	[22]
		Terpinen-4-ol (11.6; 6.0%)	
P. Terebinthus	Turkey	Terpinen-4-ol (33.7%)	[19]
		γ-Terpinene (9.3%)	
		α-Terpineol (8.1%)	
P. Lentiscus	Turkey	Terpinin-4-ol (23.9%)	[19]
		α-Terpineol (10.6%)	
		Limonene (10.6%)	
	Greek island	α-Pinene (9.4–24.9%)	[23]
		Limonene (9.0–17.8%)	
		Germacrene D (2.7–13.5%)	
		Terpinen-4-ol (6.8–10.6%)	
	Spain	α-Pinene (11.0%)	[12]
		β-Myrcene (19.0%)	
	Algeria	Terpinen-4-ol (17.3–34.7%)	[24]
		α-Terpineol (10.4–11.0%)	
		Germacrene D (8.4–15.8%)	
	Egypt	Car-3-ene (65.3%)	[14]
P. Khinjuk Stocks	Egypt	α-Pinene (18.4%)	[14]
		Sabinene (13.5%)	
		Terpinen-4-ol (12.1%)	
P. Chinensis Bunge	Egypt	(*trans*) β-Ocimene (38.8%)	[14]
		Limonene (26.5%)	
P. Palaestina Boiss.	Jordan	α-Pinene (63.1%)	[20]
		Myrcene (13.3%)	

Table 4 Seasonal variation of antioxidant activity of essential oil of leaves (male and female) of *Pistacia atlantica* using DPPH and FRAP assays

Months	IC_{50} (mg/mL)[a]		AEAC (mg/mL)[b]	
	Male	Female	Male	Female
June	20.61 ± 0.49	14.96 ± 0.35	8.39 ± 0.07	9.18 ± 0.06
July	24.95 ± 0.11	17.26 ± 0.11	4.95 ± 0.18	9.04 ± 0.08
August	25.28 ± 0.28	17.45 ± 0.57	6.74 ± 0.14	11.81 ± 0.23
September	27.87 ± 0.30	8.47 ± 0.20	5.43 ± 0.14	9.48 ± 0.06
October	27.48 ± 0.53	8.91 ± 0.44	5.66 ± 0.07	9.37 ± 0.08

[a] Using DPPH assay

[b] Using FRAP assay, values are expressed as (ascorbic acid equivalent antioxidant capacity)

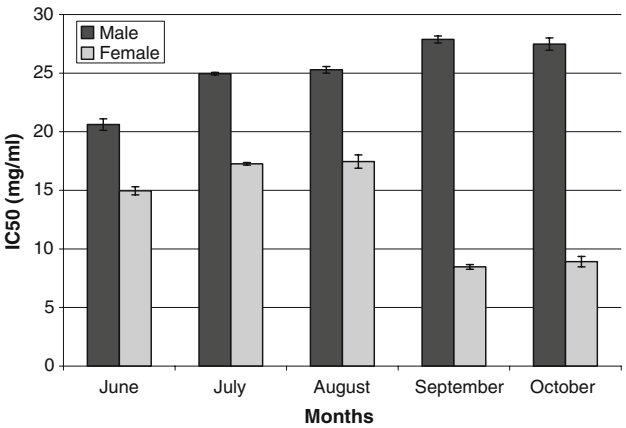

Fig. 3 Seasonal variations of the values of IC_{50} of essential oils of leaves (male and female) of *Pistacia atlantica* using DPPH assay

always less than its highest value reached in September. For the female oil, the values of AEAC are steady all over the season except for the month of August where it slowly increased to its highest value. The best antioxidant power of the oils was compared to synthetic antioxidants: BHA, BHT, Caffeic acid and Gallic acid (Table 5). The

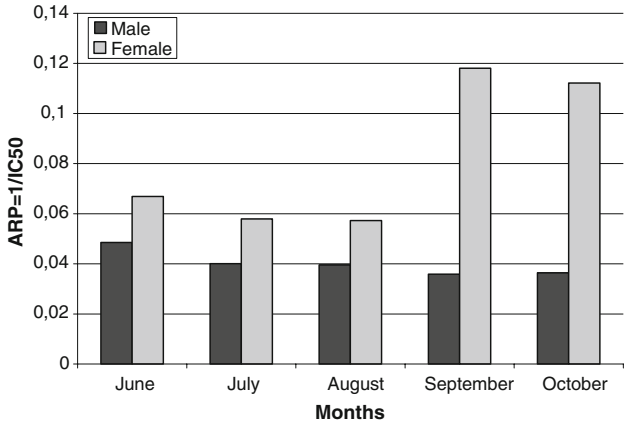

Fig. 4 Seasonal variation of antiradical power (ARP = $1/IC_{50}$) of essential oils of leaves (male and female) of *Pistacia atlantica* using DPPH assay

Table 5 Antioxidant activities of syntactic antioxidants using DPPH and FRAP assays

Antioxidant of reference	IC_{50} (μg/mL)[a]	AEAC (mg/mL)[b]
BHA	7.12 ± 0.14	0.421 ± 0.003
BHT	6.29 ± 0.17	0.373 ± 0.020
Ascorbic acid	4.48 ± 0.08	–
Caffeic acid	–	9.649 ± 0.033
Gallic acid	–	21.267 ± 0.207

[a] Using the DPPH assay

[b] Using the FRAP assay, values are expressed as (ascorbic acid equivalent antioxidant capacity)

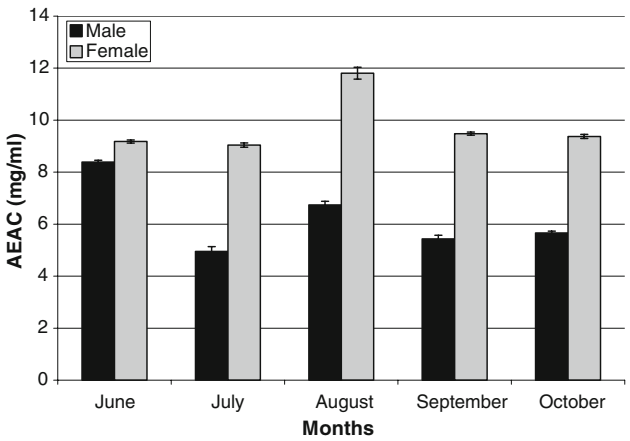

Fig. 5 Seasonal variation of the values of AEAC of essential oil of leaves (male and female) of *Pistacia atlantica* using FRAP assay

antioxidant activity of the investigated oil is higher than BHA and BHT, almost equal to caffeic acid but almost half the activity of Gallic acid.

In general, the values obtained for both DPPH and FRAP assays suggest that the essential oil of female leaves are more active than of the male ones. Finally, the data reveals the strong antioxidant activity of female oils, which are almost ten times more active than Ascorbic acid in terms of FRAP assay.

The two assays employed—DPPH and FRAP—are complementary in vitro tests of antioxidant activity. i.e. the DPPH test measures the power of scavenging free radicals in organic system environment, whereas the FRAP test measures the power of reducing of metal ions in a water system environment. Although, the results of the DPPH and FRAP tests indicates a weak potential of both male and the female essential oils to bleach the free radicals present in organic solution, they shows a strong antioxidant capacity to reduce metal ions present in water solution. Transition metals are responsible of substrate oxidation via their generation of oxidising species precursors. Since the employed tests uses different reactions mechanisms, different liquid environments and different ways of expressing results, no comparison between the results of the two tests could be done. The use of two tests has the advantage of characterising the whole potential activity of the essential oils in regard to their possible uses as antioxidant additives in food-stuffs. In order to assess the real antioxidant capacity of the essential oils a direct method involving the use of an oxidisable substrate should be employed. This direct method is based on assessing the inhibitory effect of a potentially antioxidant substance on the oxidative degradation of a substrate in a test system subjected to natural or accelerated oxidation conditions. The oxidisable substrate usually consists of individual or mixed lipids, plant proteins, fluorophores, chromophores, DNA, or fluids containing biologically active chemical species such as low-density lipoproteins (LDLs) and biological membranes.

No evident or clearly direct relations were found between the chemical compounds of the essential oils (for both male and female) and the values of their antioxidant activities. This could be due to the complexity of the essential oils content and their content evolutions during the season. In fact, some compounds of the essential oils are very actives, others are less or inactive and since the percentage of each compound during the season follows a different way of evolution (by increasing or decreasing) it is very difficult to assess or predict the total final activity of the essential oils. A high increase of the percentage of some compounds with weak or inactive antioxidants capacities will not affect considerably the total final measured activity. Moreover, the composition evolution in two opposite ways simultaneously for two compounds characterised by high individual activities could lead to the compensatory effect of the final activity. It is important to mention that the interpretation could be more complex if synergistic and the antagonistic effects took

place. In order to determine the components responsible for the high activity, another study must be conducted which would aim at the isolation of components of the essential oils. Based on the results of antioxidant activities found herein, and the fact that the female essential oils are rich in δ-3-carene, which is not known for its high activity, it could be likely that some components present in low percentages are responsible of the relatively higher activity of female essential oils in comparison to male essential oils.

Conclusion

This current phytochemical investigation shows that there was a major difference in composition between male and female leaves essential oils. Furthermore, their compositions changes during the growing season. The essential oils were characterised by their relatively low yields. The δ-3-carene monoterpene compound was the only major component of the female essential oil. The main components of the male essential oil were α-pinene/α-thujene, spathulenol and bicyclogermacrene. The variation of the composition during the growing season showed that most of the main components of the oils reach theirs highest values in September.

Although the result of the DPPH assay shows that the essential oils have a low capacity of bleaching free radicals, the values of FRAP assay were very interesting, especially for the female leaves essential oils. It is recommended to harvest the leaves from the female trees in August, the month where the essential oil exhibits its highest activity in the FRAP assay. This important antioxidant activity reveals the promising potential of the essential oil of *Pistacia atlantica* leaves as a source of natural antioxidants.

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