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# Chemotypes of *Pistacia atlantica* Leaf Essential Oils from Algeria

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The essential oils obtained by hydrodistillation of *Pistacia atlantica* Desf. leaves collected from different regions of Algeria were analyzed by GC and GC-MS. The essential oil was rich in monoterpenes and oxygenated sesquiterpenes. The major components were α-pinene (0.0-67%), δ-3-carene (0.0-56%), spathulenol (0.5-22%), camphene (0.0-21%), terpinen-4-ol (0.0-16%) and β-pinene (0.0-13%). Among the various components identified, twenty were used for statistical analyses. The result of principal component analysis (PCA) showed the occurrence of three chemotypes: a δ-3-carene chemotype (16.4-56.2%), a terpinen-4-ol chemotype (10.8-16.0%) and an α-pinene/camphene chemotype (10.9-66.6% / 3.8-20.9%). It was found that the essential oil from female plants (δ-3-carene chemotype) could be easily differentiated from the two other chemotypes corresponding to male trees.

**Keywords:** *Pistacia atlantica*, Leaves, Essential oil, Composition, Chemotype, Principal component analysis.

The genus *Pistacia* (Anacardiaceae) is widely distributed in the Mediterranean area [1]. *P. atlantica* Desf. is a tree mainly located in North Africa, which can reach over 15 m in height and grows in arid and semi-arid areas [2]. The fruits smell like mastic and are sometimes used in Lesvos Island (Greece) to flavor the alcoholic drink called “raki”. These fruits are called “tsikuda” in Lesvos and are also sometimes chewed by the local people (for mouth flavoring). The fruits of *P. atlantica* are also used for tanning and as fodder for cattle. They contain oil, which is used for soap making. From the bark of the wood, a resin is collected for lacquer production and it is also used in popular medicine as an antiseptic for wounds [3]. In Iran, the oleoresin of *P. atlantica* var. *mutica* is a popular naturally-occurring chewing gum and has been used traditionally in the treatment of peptic ulcers [4]. In Morocco, *P. atlantica* is the source of mastic gum, an exudate which strengthens gums, deodorizes breath and combats coughs, chills and stomach diseases [5]. In Jordan, *P. atlantica* is one of the trees widely recommended by herbalists and used for its hypoglycemic activity [6]. Moreover, the galls are used as an embalming ingredient by rural inhabitants. The trees are also known in Arabic as "Butom" and are edible and sold in markets.

Previous studies on *P. atlantica* have dealt with flavonoids [7], fatty acids [8-11], triacylglycerol [12], and the chemical composition of the oleoresin [4,13,14]. Recently, Yousfi *et al.* [15] have isolated a new hispolone derivative from the phenolic extracts.

The chemical compositions of the essential oils of the leaves of *P. atlantica* from various origins (Greece, Morocco, and Algeria) [3,16,17] show a large variability in their chemical composition, which indicates the existence of chemotypes in this species. In addition, a recent work [3] mentions a difference in the composition of the oils from male and female *P. atlantica*. Taking into account these considerations, using Principal Component Analysis (PCA), we have checked the occurrence of various chemotypes for the essential oil from *P. atlantica* leaves (male and female) from different locations in Algeria.

*P. atlantica* male and female trees ($n$ = 40) were randomly chosen from three locations: Aïn-Oussera, Laghouat and Hassi R'mel. The oils, prepared by hydrodistillation (Clevenger apparatus) of air-dried leaves of male and female trees, were obtained in yields of 0.09 to 0.13%, v/w, as pale-yellow to light-brown colored liquids with an aromatic-spicy odor. Forty-seven compounds were identified (Table 1). The essential oil was rich in monoterpenes and oxygenated sesquiterpenes. The main components were α-pinene (0.0-67%), δ-3-carene (0.0-56%), spathulenol (0.5-22%), camphene (0.0-21%), terpinen-4-ol (0.0-16%) and β-pinene (0.0-13%). The chemical compositions of the essential oils presented a large variability in the main components. This supposes the existence of different chemotypes within the same species. Taking this into account, we proceeded to multivariate statistical analysis.

The data set, composed of 40 samples and 20 variables (main compounds), were subjected to multivariate statistical analysis for chemotype determination. Loading factors for principal axes F1 and F2 are given in Figure 1. Axis F1, which represents 33% of the total information, is highly negatively correlated with α-pinene, β-pinene, camphene, tricyclene, limonene, bornyl acetate and positively correlated with β-phellandrene, *p*-cymene, γ-terpinene, terpinen-4-ol, and myrcene. This axis separates the samples into two point clouds (Figure 2). The F2 Axis, which represents 19% of the total information, is positively correlated with δ-3-carene, bicyclogermacrene, and limonene, and negatively correlated with *p*-cymene, γ-terpinene, and terpinen-4-ol. This axis separates the samples into two new point clouds (Figure 2). The factor loading of the 20 variables on axes F1 and F2 (circle of correlations), using PCA of the chemical composition obtained by gas chromatography of the 40 essential oil samples represented in Figure 1, shows strong correlations with some components of the essential oils (Table 2). Tricyclene is strongly correlated with camphene (R=0.998) and highly correlated with both β-pinene (R=0.824) and α-pinene (R=0.717). On the other hand, camphene is highly correlated with both β-pinene (R=0.818) and α-pinene (R=0.713). γ- Terpinene is highly correlated with β-phellandrene (R=0.880), *p*-cymene (R=0.805) and terpinen-4-ol (R=0.958). At the same time, β-phellandrene is highly correlated with terpinen-4-ol (R=0.845) and *p*-cymene (R=0.786). Finally, terpinen-4-ol and *p*-cymene are also highly correlated (R=0.857). It is very important to note that the correlations are mostly positives between the selected components and the essential oils. This observation indicates possible biosynthetic pathways of these compounds.

Taking into account the chemical content range, we can consider three groups or chemotypes, as shown in Figure 2. Table 3 gives the data for each chemotype. Chemotype I (or δ-3-carene chemotype) is characterized by a high δ-3-carene percentage (16.4-56.2%). Chemotype II (or terpinen-4-ol chemotype) is characterized by the highest content of this compound (10.8-16.0%), *p*-cymene (2.7-10.2%), and α-pinene (6.2-15.8%). Chemotype III (or α-pinene/camphene chemotype) differs from the other ones by a high percentage of α-pinene (10.9-66.6%), camphene (3.8-20.9%) and β-pinene (2.5-13.1%). Although, spathulenol is considered as one of the main components, it has practically no effect on chemotype differentiations.

It was noticed that the α-pinene/camphene chemotype could be separated into two very close subgroups SG1 and SG2, which are separated and differentiated by the second axis F2. The subgroup SG1, which is located above the F1 axis, is characterized by a relatively higher percentage of bicyclogermacrene (0.0-9.9%) compared with the SG2 subgroup.

It is very interesting to mention that some chemical components present in low relative percentages could be able to differentiate the essential oil chemotypes. This is the case with β-phellandrene and γ-terpinene. In fact, examination of the percentage ranges for the three chemotypes shows the absence of β-phellandrene in the essential oil of the α-pinene/ camphene chemotype and the absence of γ-terpinene in the δ-3-carene chemotype. Moreover, the δ-3-carene and the α-pinene/camphene chemotypes are characterized by the quasi-absence of β-phellandrene and γ-terpinene, respectively. On the other hand, the terpinen-4-ol chemotype is characterized by the presence of both β-phellandrene and γ-terpinene in low amounts (Table 3).

Comparison between the composition of *P. atlantica* leaf essential oils from Algeria with those reported in the literature [3,16,17] (Table 4) reveals the existence of both significant similarities and differences for the main component percentages. Terpinen-4-ol seems to be the major component only for essential oils from Greece and Morocco (10-20%). The essential oil from Algeria is quite different from those of all other origins, since it is distinguished by the presence of α- and β-pinene, which are not present as main components in the oils of other origins. Another major difference is that Greek oils are characterized by a high *p*-mentha-1(7),8-diene percentage (the main component) for the male essential oil and by myrcene for the female essential oil. The latter observation, which expresses the difference between the male and female essential oils, has been taken into consideration in our study. The essential oil from Morocco is characterized by elemol as its major component, which is not reported elsewhere. This

**Table 1**: Compound identification of *P. atlantica* leaf essential oil from Algeria.

| Compounds | LRI[a] | Min.[b] | Max.[b] | Mean[c] | s.d. | Identification[d] |
|---|---|---|---|---|---|---|
| Tricyclene | 1016 | 0.0 | 6.4 | 1.9 | 1.7 | MS, RI |
| α-Pinene | 1028 | 0.0 | 66.6 | 20.8 | 15.6 | MS, RI |
| Camphene | 1053 | 0.0 | 20.9 | 6.3 | 5.3 | MS, RI |
| β-Pinene | 1118 | 0.0 | 13.1 | 4.4 | 3.0 | MS, RI |
| Verbenene | 1129 | 0.0 | 2.9 | 0.7 | 0.9 | MS, RI |
| δ-3-Carene | 1160 | 0.0 | 56.2 | 5.3 | 14.8 | MS, RI |
| Myrcene | 1163 | 0.0 | 1.5 | 0.2 | 0.4 | MS, RI, AS |
| α-Phellandrene | 1174 | 0.0 | 0.8 | 0.2 | 0.3 | MS, RI |
| α-Terpinene | 1189 | 0.0 | 3.9 | 0.8 | 1.1 | MS, RI |
| Limonene | 1209 | 0.0 | 1.9 | 0.9 | 0.4 | MS, RI, AS |
| β-Phellandrene | 1219 | 0.0 | 1.2 | 0.4 | 0.4 | MS, RI |
| *trans*-2-Hexenal | 1235 | 0.0 | 2.1 | 0.5 | 0.6 | MS, RI |
| γ-Terpinene | 1255 | 0.0 | 6.7 | 1.4 | 2.2 | MS, RI |
| *p*-Cymene | 1282 | 0.0 | 10.2 | 2.3 | 3.1 | MS, RI |
| α-Terpinolene | 1293 | 0.0 | 1.9 | 0.6 | 0.6 | MS, RI |
| 6-Methyl-5-hepten-2-one | 1336 | 0.0 | 1.8 | 0.2 | 0.4 | MS, RI |
| (*E*)-3-Hexen-1,ol | 1351 | 0.0 | 1.2 | 0.2 | 0.3 | MS, RI |
| *trans*-2-Hexenol | 1361 | 0.0 | 0.5 | 0.0 | 0.1 | MS, RI |
| (*Z*)-3-Hexen-1,ol | 1396 | 0.0 | 1.5 | 0.2 | 0.4 | MS, RI |
| 2-Hexen-1-ol | 1406 | 0.0 | 0.9 | 0.1 | 0.2 | MS, RI |
| β-Thujone | 1456 | 0.0 | 2.3 | 0.8 | 0.6 | MS, RI |
| Bornylene | 1540 | 0.0 | 3.1 | 1,0 | 0.8 | MS, RI |
| Linalool | 1557 | 0.0 | 2.6 | 0.5 | 0.6 | MS, RI, AS |
| Bornyl acetate | 1595 | 0.0 | 7.2 | 2.3 | 1.8 | MS, RI |
| Camphene hydrate | 1604 | 0.0 | 0.5 | 0.0 | 0.1 | MS, RI |
| Terpinen-4-ol | 1617 | 0.0 | 16.0 | 4.7 | 5.9 | MS, RI, AS |
| Aromadendrene | 1658 | 0.0 | 1.7 | 0.2 | 0.5 | MS, RI, AS |
| Alloaromadendrene | 1657 | 0.0 | 1.7 | 0.4 | 0.5 | MS, RI, AS |
| (*E*)-Pinocarveol | 1671 | 0.0 | 2.0 | 0.8 | 0.5 | MS, RI |
| Ledene | 1678 | 0.0 | 0.8 | 0.0 | 0.1 | MS, RI |
| Linalyl propionate | 1700 | 0.6 | 3.5 | 1.9 | 0.8 | MS, RI |
| α-Terpineol | 1712 | 0.7 | 6.5 | 3.0 | 1.3 | MS, RI, AS |
| α-Terpenyl acetate | 1722 | 0.0 | 7.0 | 2.0 | 1.9 | MS, RI |
| Bicyclogermacrene | 1750 | 0.0 | 9.9 | 1.6 | 2.3 | MS, RI |
| Germacrene B | 1846 | 0.0 | 1.2 | 0.3 | 0.4 | MS, RI |
| Geranyl acetone | 1869 | 0.0 | 3.2 | 1.1 | 0.8 | MS, RI |
| Palustrol | 1960 | 0.0 | 0.6 | 0.1 | 0.2 | MS, RI |
| Epiglobulol | 2028 | 0.0 | 2.1 | 0.6 | 0.6 | MS, RI |
| Ledol | 2050 | 0.0 | 4.8 | 0.7 | 0.9 | MS, RI, AS |
| Globulol | 2070 | 0.0 | 5.5 | 1.5 | 1.2 | MS, RI, AS |
| Viridiflorol | 2100 | 0.0 | 2.4 | 0.4 | 0.6 | MS, RI, AS |
| Spathulenol | 2143 | 0.5 | 22.1 | 7.4 | 6.0 | MS, RI |
| γ-Eudesmol | 2186 | 0.0 | 4.3 | 1.3 | 0.9 | MS, RI |
| Isospathulenol | 2245 | 0,5 | 6.9 | 2.6 | 1.6 | MS, RI |
| Phytol | 2623 | 0.0 | 1.5 | 0.3 | 0.4 | MS, RI, AS |
| Myristic acid | 2663 | 0.0 | 2.6 | 0.2 | 0.5 | MS, RI |
| Palmitic acid | 2920 | 0.0 | 11.3 | 1.5 | 1.9 | MS, RI |

[a] Experimental linear retention indices determined on the UB-Wax column. [b] Relative area percentage. [c] Mean of 40 samples.
[d] RI: Retention indices on UB-wax column. MS: mass spectrometry. AS: Retention indices of pure authentic samples.

present study confirms the presence of different chemotypes for the essential oil of *P. atlantica*. In fact, our results are very close to those of previous works since the α-pinene chemotype has some similarity to the essential oil reported by Mecherara-Idjeri [17]; the terpinen-4-chemotype has some similarities to the rest of the essential oils reported by others [3,16]. As a result, this study must be extended to larger areas and locations to ensure the identification of all possible existing chemotypes of *P. atlantica*.

## Experimental

***Plant material:*** Fresh leaves from male and female *P. atlantica* trees were collected at the end of October 2007 from three different Algerian areas: Aïn-Oussera, Laghouat and Hassi R'mel (respectively located at 200, 400 and 500 km South of Algiers).

***Preparation of samples:*** Forty samples were used. The samples of leaves were air-dried in the shade at room temperature. The oils were obtained by hydrodistillation using a Clevenger apparatus for up to 8 h. The oils, obtained in yields of 0.09-0.13% v/w, were dried over anhydrous sodium sulfate, filtered and stored at +4°C until analysis.

***Gas chromatography (GC) analysis:*** A CP-Varian 3800 gas chromatograph was used with a flame ionization detector (FID), and a UB-Wax fused silica capillary column (60 m × 0.32 mm, 0.25 µm film thickness). Oven temperature was programmed from 50°C 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°C and 260°C, respectively. Helium was the carrier gas at a flow rate of 1 mL/min.

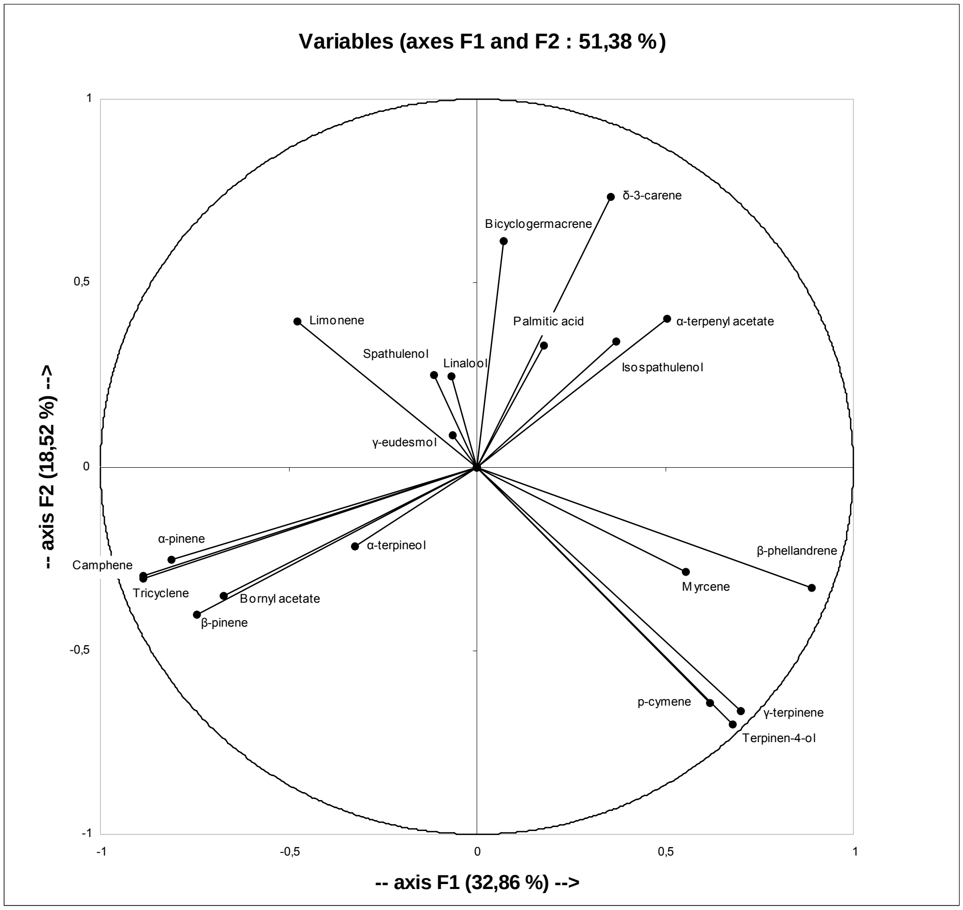


**Figure 1**: Factor loading of 20 variables on axes F1 and F2 using Principal Component Analysis of the 40 *P. atlantica* essential oil samples from Algeria.

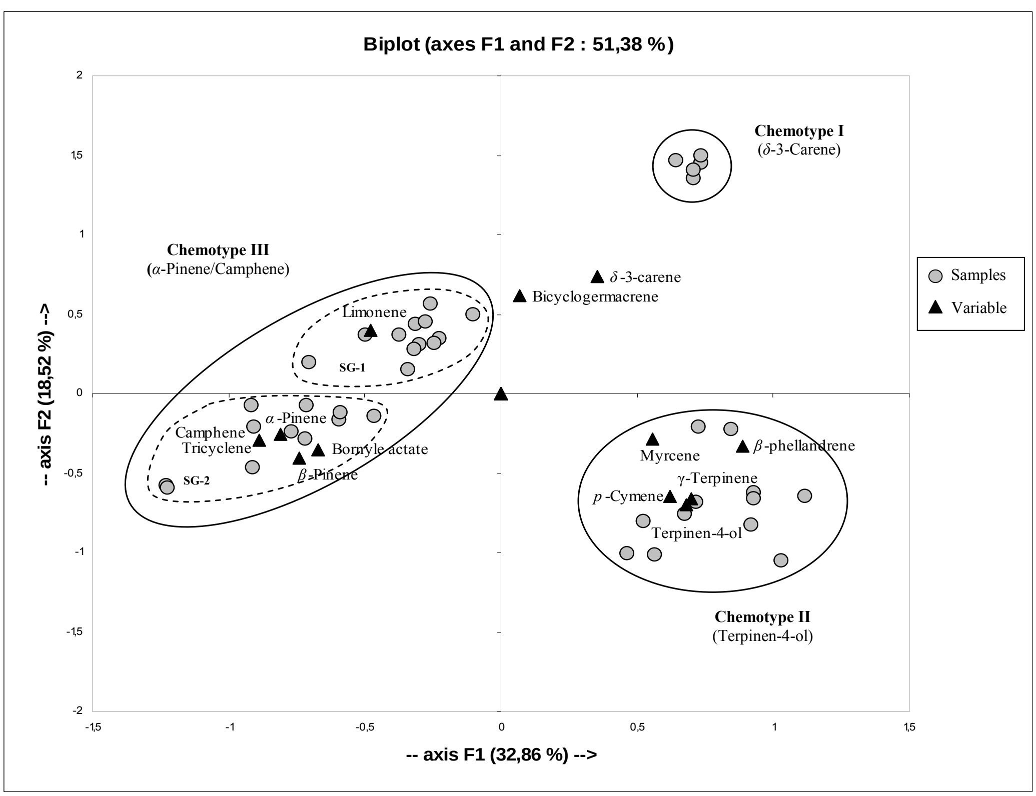


**Figure 2**: Two dimensional plot on axes F1 and F2 of the essential oil samples of *P. atlantica* from Algeria using Principal Component Analysis.

**Table 2**: Correlation coefficients obtained between some components of the essential oils of *P. atlantica* using Principal Component Analysis.

| | Tricyclene | α-Pinene | Camphene | β-Pinene | β-Phellandrene | γ-Terpinene | *p*-Cymene |
|---|---|---|---|---|---|---|---|
| α-Pinene | **0.717** | | | | | | |
| Camphene | **0.998** | **0.713** | | | | | |
| β-Pinene | **0.824** | 0.666 | **0.818** | | | | |
| β-Phellandrene | -0.614 | -0.663 | -0.619 | -0.471 | | | |
| γ-Terpinene | -0.408 | -0.419 | -0.412 | -0.209 | **0.880** | | |
| *p*-Cymene | -0.328 | -0.394 | -0.337 | -0.197 | **0.786** | **0.805** | |
| Terpinen-4-ol | -0.384 | -0.374 | -0.389 | -0.206 | **0.845** | **0.958** | **0.857** |

**Table 3**: Mean and range of three *P. atlantica* essential oil chemotypes from Algeria.

| **Compounds** | **δ-3-carene chemotype[a]** | | | | **Terpinen-4-ol chemotype[b]** | | | | **α-pinene/camphene chemotype[c]** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Min.** | **Max.** | **mean** | **s.d.** | **Min.** | **Max.** | **mean** | **s.d.** | **Min.** | **Max.** | **mean** | **s.d.** |
| Tricyclene | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 1.7 | 0.8 | 0.6 | 1.1 | 6.4 | 2.9 | 1.5 |
| **α-Pinene** | 0.0 | 1.1 | 0.8 | 0.4 | 6.2 | 15.8 | 10.5 | 3.3 | 10.9 | 66.6 | **31.0** | 12.7 |
| **Camphene** | 0.0 | 0.6 | 0.4 | 0.2 | 0.8 | 5.6 | 2.8 | 1.7 | 3.8 | 20.9 | **9.4** | 4.7 |
| β-Pinene | 0.0 | 0.4 | 0.1 | 0.2 | 1.9 | 4.9 | 3.3 | 0.9 | 2.5 | 13.1 | 5.9 | 2.9 |
| **δ-3-Carene** | 16.4 | 56.2 | **41.6** | 15.2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 2.3 | 0.1 | 0.5 |
| Myrcene | 0.0 | 0.6 | 0.3 | 0.3 | 0.0 | 1.5 | 0.5 | 0.5 | 0.0 | 0.9 | 0.1 | 0.2 |
| Limonene | 0.6 | 1.9 | 1.4 | 0.5 | 0.0 | 1.1 | 0.6 | 0.4 | 0.6 | 1.6 | 1.1 | 0.3 |
| β-Phellandrene | 0.4 | 0.8 | 0.6 | 0.2 | 0.7 | 1.3 | 1.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 |
| γ-Terpinene | 0.0 | 0.0 | 0.0 | 0.0 | 3.2 | 6.9 | 5.1 | 1.2 | 0.0 | 0.4 | 0.0 | 0.1 |
| *p*-Cymene | 0.0 | 0.6 | 0.4 | 0.2 | 2.7 | 10.2 | 6.3 | 2.6 | 0.0 | 1.2 | 0.4 | 0.3 |
| Linalool | 0.0 | 1.3 | 0.9 | 0.6 | 0.0 | 0.8 | 0.3 | 0.3 | 0.0 | 2.6 | 0.4 | 0.7 |
| Bornyl acetate | 0.0 | 1.1 | 0.3 | 0.5 | 0.0 | 3.2 | 1.6 | 1.0 | 0.0 | 7.2 | 3.2 | 1.8 |
| **Terpinen-4-ol** | 0.0 | 0.7 | 0.3 | 0.3 | 10.8 | 16.0 | **13.9** | 1.8 | 0.0 | 7.2 | 1.1 | 1.6 |
| α-Terpineol | 0.7 | 1.7 | 1.2 | 0.4 | 1.8 | 4.5 | 2.8 | 0.8 | 1.9 | 6.5 | 3.4 | 1.2 |
| α-Terpenyl acetate | 3.6 | 7.0 | 5.6 | 1.4 | 0.5 | 6.3 | 2.3 | 1.9 | 0.0 | 2.7 | 1.1 | 0.8 |
| Bicyclogermacrene | 2.5 | 4.4 | 3.9 | 0.8 | 0.0 | 1.6 | 0.4 | 0.6 | 0.0 | 9.9 | 1.7 | 2.7 |
| Spathulenol | 2.7 | 5.0 | 3.9 | 0.9 | 1.8 | 14.5 | 5.1 | 3.8 | 0.5 | 22.1 | 9.2 | 7.4 |
| γ-Eudesmol | 0.5 | 1.6 | 1.0 | 0.5 | 0.0 | 2.2 | 1.2 | 0.6 | 0.0 | 4.3 | 1.4 | 1.1 |
| Isospathulenol | 1.8 | 6.2 | 3.7 | 1.7 | 0.7 | 5.6 | 2.8 | 1.5 | 0.5 | 6.9 | 2.2 | 1.5 |
| Palmitic acid | 0.0 | 11.3 | 2.8 | 4.8 | 0.0 | 2.8 | 1.2 | 0.7 | 0.0 | 4.5 | 1.2 | 1.1 |

[a] 5 samples, [b] 12 samples, [c] 23 samples
s.d. standard deviation

**Table 4**: Range of main chemical components of *P. atlantica* leaf essential oils from different origins.

| **Origin of country** | **Mains components** | **Percentage** | **Ref** |
|---|---|---|---|
| Greece (male) | *p*-Menta-1(7),8-diene<br>Terpinen-4-ol | (41.1%)<br>(17.3%) | [3] |
| Greece (female) | Myrcene<br>Terpinen-4-ol | (17.8 ; 24.8%)<br>(11.6 ; 6.0%) | [3] |
| Morocco | Terpinen-4-ol<br>Elemol | (21.7%)<br>(20.0%) | [16] |
| Algeria | α-Pinene<br>β-Pinene | (32.6-54.7%)<br>(8.0-20.2%) | [17] |

***Gas chromatography-mass spectrometric (GC-MS) analysis:*** The GC-MS analyses were performed on an Agilent 6890 GC machine equipped with a capillary column HP5MS (30 m × 0.25 mm, 0.25 µm film thickness) and coupled with a quadripole CMSD 5973 detector in electron ionization mode (EI 70 eV). 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. Column temperature was initially at 60°C, held for 2 min, then gradually increased to 125°C at a 2°C/min rate, held for 2 min, and finally increased to 220°C at 5°C/min and held for 2 min. Diluted samples (1:100 v/v, in ethanol) of 1 µL were injected manually using splitless mode.

***Compound identifications:*** Linear retention indices relative to linear homologous *n*-alkanes ($C_8$–$C_{40}$) were calculated. The identifications of the components were based on the comparison of their MS 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 literature values and by co-injections of some pure authentic samples.

***Multivariate statistical analyses:*** Principal component analysis (PCA) was performed using the data set composed of 40 oil samples and 20 variables (20 main compounds determined on UB-Wax capillary column). Results were obtained using XLSTAT 7.5.2 program.

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