Choukri Tefiani, Ali Riazi, Boumediene Belbachir, Hicham Lahmar, Smail Aazza, Ana Cristina Figueiredo, Maria Graça Miguel*

*Ammoides pusilla* (Brot.) Breistr. from Algeria: Effect of harvesting place and plant part (leaves and flowers) on the essential oils chemical composition and antioxidant activity

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**Abstract:** The chemical variability and antioxidant activity of the flower and leaf essential oils (EOs) of *Ammoides pusilla*, collected at Algeria was evaluated. The EOs were isolated by hydrodistillation and analyzed by Gas Chromatography (GC) and Gas Chromatography-Mass Spectrometry (GC-MS). Antioxidant activity was assessed by DPPH, ABTS, Reducing Power and TBARS assays. Oxygen-containing monoterpenes (54-77%) dominated all but one *A. pusilla* leaf EOs, and in two flower EOs (53% both). Thymol dominated in practically all leaf and flowers EOs, but cumin alcohol, *p*-cymene and limonene attained also relatively high percentages in some EOs. A strong negative correlation (*p*<0.01) between IC_{50} values of ABTS, DPPH, and hydroxyl scavenging activity and the percentages of *p*-cymene and cumin alcohol present in EOs were observed, showing that higher levels of these monoterpenes were responsible for the best activities found. In spite of this finding, the antagonism and/or synergism between EO components must be taken into account, since the EO activity can only be considered as a whole. Moreover, given the EOs chemical variability their use as antioxidants, should be preceded by their chemical evaluation.

**Keywords:** Radical scavenging, lipid peroxidation, thymol, cumin alcohol, *p*-cymene

1 Introduction

Ajowan, *Ammoides pusilla* (Brot.) Breistr. is an annual aromatic herbaceous species belonging to the Apiaceae family [1]. In Algeria, this species is known as *Noukha*. In the North African countries, the same species is known as *Nabta*, *Ridjl El-Ghorab* and *Gazar Ech-Cheytan*, and is known for its digestive properties [2]. In addition, it is used in traditional medicine as infusion for treating headache, fever, flu and diarrhoea, or as a compress alone or soaked in alcohol and mixed with henna for treating mental debility of children.

*A. pusilla* infusions, solvent extracts, as well essential oils (EOs), have shown diverse biological properties, namely antioxidant [2,3], and antimicrobial activities [1,2,4,5,6]. The antiproliferative activity of *A. pusilla* EOs against human acute monocytic leukaemia cell line (THP-1) [1], and antidiabetic activity of water extracts in Wistar rats [7,8], were also reported.

There are diverse factors that determine the chemical variability of volatile components and essential oils and which include physiological variations, environmental conditions, genetic factors and evolution, geographic variations, among others [9]. The chemical variability of essential oils is responsible for the variability of biological properties including antioxidant activity [10].
In the course of our ongoing work on the biological activities of *A. pusilla* from Algeria, the main goal of the present work was to study the importance of plant part used and the harvesting places on the chemical composition and antioxidant activity of the essential oils.

### 2 Material and Methods

#### 2.1 Material

Plants were harvested in May-June 2013 in different places of Algeria (Table 1), leaves and flowers separated and dried at room temperature in darkness. Plants were identified by Prof. Bouazzza from Tlemcen University, but no voucher number was kept at that date.

#### 2.2 Isolation of Essential Oils

Dried flower and leaves of *A. pusilla* (100 g) were grounded to a homogeneous powder and placed in a 2 L round bottom flask. An adequate amount of distilled water was added so that the plant material to be completely immersed. The hydrodistillation was conducted using a Clevenger-type apparatus according to the European Pharmacopoeia method [11] for 3 h.

#### 2.3 Analysis of essential oils

Gas chromatography (GC) and GC–mass spectrometry (GC–MS) analyses were performed as previously detailed [1,12,13] and the percentage composition of the EOs was computed by the normalization method as reported previously [1,12].

#### 2.4 Antioxidant activity

##### 2.4.1 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity

Free radical-scavenging activities of essential oils were measured using DPPH as previously described [12]. Briefly, 50 μL of a methanolic stock solution of each EO at different concentrations was placed in a cuvette, and 2 mL 60 μM methanolic solution of DPPH was added. Absorbance measurements were made at 517 nm after 60 min of reaction at room temperature. Absorption of a blank sample containing the same amount of methanol and DPPH solution acted as the negative control. All determinations were performed in triplicate. The percentage inhibition of the DPPH radical by the samples was calculated according to the formula: % Inhibition = [(A₀ - A₁) / A₀] x 100; where A₀ is the absorption of the blank sample (t = 0 min) and A₁ is the absorption of the tested oil (t = 60 min). The EO concentration providing 50% inhibition (IC₅₀) was obtained by plotting the inhibition percentage against EOs concentrations.

##### 2.4.2 2,2’-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) free radical-scavenging activity

The determination of ABTS radical scavenging was carried out as reported by Aazza et al. [14]. The sample concentration providing 50% inhibition (IC₅₀) was obtained by plotting the inhibition percentage against EOs concentrations.

##### 2.4.3 Reducing Power

The reductive potential of the essential oils was determined according to Aazza et al. [15]. Each methanolic stock solution of each EO at different concentrations was mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide [K₃Fe(CN)₆] (2.5 mL, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 mL) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged for 10 min at 3000 rpm. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and FeCl₃ (0.5 mL, 0.1%), and the absorbance was measured at 700 nm. The assay was carried out in triplicate.

### Table 1: *Ammoides pusilla* collection sites in Algeria and corresponding codes.

| Collection places | Code | Latitude       | Longitude       | Altitude (m) |
|-------------------|------|----------------|-----------------|--------------|
| Sidi Safi         | SS   | 35°16'28.82"   | 1°19'04.05"     | 200          |
| Terni             | T    | 34°47'21.69"   | 1°20'38.23"     | 1336         |
| Ouzidane          | O    | 34°56'19.51"   | 1°16'23.92"     | 540          |
| Beni Snous        | BS   | 34°39'18.90"   | 1°31'59.11"     | 732          |
| Ain Fezza         | AF   | 34°52'50.49"   | 1°14'05.04"     | 881          |
| Fehoul            | F    | 35°07'05.50"   | 1°16'43.44"     | 322          |
| Boedj Arima       | BA   | 35°05'37.51"   | 1°34'16.83"     | 218          |
| Ain Kihal         | AK   | 35°12'52.41"   | 1°11'45.18"     | 438          |
2.4.4 Thiobarbituric acid reactive species (TBARS)

Essential oils ability to inhibit malondialdehyde formation, and therefore lipid peroxidation, was determined by using a modified thiobarbituric acid reactive species (TBARS) assay as previously described [15]. Five hundred microlitres of egg yolk homogenates 10% (w/v) and methanolic solution of EO (0.1 mL) were added to a test tube and made up to 1 mL with distilled water. Then, 20% acetic acid (1.5 mL, pH 3.5) and 0.8% (w/v) thiobarbituric acid (TBA, 1.5 mL) in 1.1% (w/v) sodium dodecyl sulphate (SDS) were added. The resulting mixture was vortexed and heated at 95 °C for 60 min. After cooling, at room temperature, 1-butanol (5 mL) was added to each tube; the contents of the tubes were stirred and centrifuged at 3000 rpm for 10 min. The absorbance of the organic upper layer was measured at 532 nm. The antioxidant capacity measurements were carried out in triplicate. All of the values were based on the percentage antioxidant index (AI%), whereby the control was completely peroxidized and each EO demonstrated a degree of change; the percentage inhibition was calculated using the formula 
\[
\frac{(A_0 - A_1)}{A_0} \times 100,
\]
where \(A_0\) is the absorbance value of the fully oxidized control and \(A_1\) is the absorbance of the test EO. The percentage antioxidant index was plotted against the concentrations of samples or standards and IC\(_{50}\) values were determined (concentration of EO to prevent 50% of lipid oxidation).

2.5 Statistical analysis

Statistical analysis was performed with the Statistical Package for the Social Sciences (SPSS) 23.0 software (SPSS Inc., Chicago, IL). Statistical comparisons were made with one-way analysis of variance followed by Tukey multiple comparisons. The level of significance was set at \(P < 0.05\). Correlations between major compounds of the essential oils percentages and antioxidant activity were achieved by Spearman correlation coefficient (\(r\)) at a significance level of 95% \((P < 0.01)\). Paired Student t test were used in some tests to determine differences at 5% and 1% significance.

3 Results and discussion

3.1 Essential oil composition

Essential oils (EOs) yields and identified essential oil (EO) components are listed in Table 2 in the elution order on the DB-1 column. Flowers (2.4-3.6 %, v/w) yielded higher percentages of EOs than leaves (1.1-3.0 %), whatever the geographic origin of plants. The yield percentages are in the same range of values of those reported previously by Tefiani et al. [1], particularly those of flowers. Twenty-six compounds were identified in \(A.\) pusilla.

Oxygen-containing monoterpenes dominated (54-77%) in practically all \(A.\) pusilla leaf EOs, with the exception of Ain Kihal sample. In this case, monoterpene hydrocarbons predominated (65%). In flower EOs, there were two samples in which this group of terpenes predominated, Terni (53%) and Fehoul (53%). In the remaining samples, oxygen-containing monoterpenes prevailed (53-75%) (Table 2).

Thymol (Fig 1) dominated in Sidi (40%), Terni (29%), Ouzidane (50%), Ain Fezza (60%), Fahoul (53%) and Bordj Ariona (50%) leaf EOs. Cumin alcohol (Fig. 1) predominated in Beni Snous leaf EOs (49%). p-Cymene (35%) (Fig. 1), along with limonene (26%) (Fig. 1) dominated in Ain Kihal sample EO (Table 2).

In the flower EOs, thymol dominated in Terni (31%), Ouzidane (42%), Ain Fezza (46%), Fahoul (39%), Bordj Arima (58%), and Ain Kihal (42%) samples, whereas in Beni Snous sample EO, cumin alcohol was the most important. Cumin alcohol and thymol showed similar percentages (36 and 36%, respectively) in Sidi Safi flower EO (Table 2).

In all samples, p-cymene and limonene were also detected in relative high percentages (>5%). The components detected in both leaf and flower EOs were the same, nevertheless in some cases the percentages of some compounds varied greatly depending on the region, albeit no correlation could be found between latitude, longitude or altitude of collection zones (Table 1) and percentages of the major compounds detected in all EOs (Table 2). For example, flower EO from Terni had much lower percentage of cumin alcohol (14%) than leaf EO (24%). Other examples include the lower percentages of thymol in flower EOs from Ain Fezza (46%) and Fahoul (39%) than in leaf EOs (60 and 53%, respectively). In some cases, the percentage of oxygen-containing monoterpenes varied greatly among samples, but no correlation could be found between the percentages of the major compounds in all EOs and the geographic origin of plants.
Table 2: Percentage composition of the essential oils isolated from the leaves and flowers of *Ammoides pusilla* populations from different collection sites in Algeria.

| Components | RI   | RI*  | Leaf | Flower |
|------------|------|------|------|--------|
|            |      |      | SS   | T | O | BS | AF | F | BA | AK | SS | T | O | BS | AF | F | BA | AK |
| α-Thujene  | 924  | 929  | t    | 0.1 | 0.1 | 0.2 | t  | t  | t  | 0.3 | t  | 0.3 | 0.2 | 0.2 | 0.1 | 0.1 | t  | t  |
| α-Pinene   | 930  | 937  | 0.3  | 0.6 | 0.5 | 0.2 | 0.2 | 0.2 | 0.2 | 0.8 | 0.2 | 1.2 | 0.6 | 0.7 | 0.8 | 0.4 | 0.2 | 0.2 |
| Sabinene   | 958  | 961  | t    | 0.1 | 0.1 | 0.1 | t  | t  | t  | 0.1 | t  | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | t  | t  |
| β-Pinene   | 963  | 963  | t    | 0.1 | 0.1 | 0.1 | t  | t  | t  | 0.1 | t  | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | t  | t  |
| β-Myrcele  | 975  | 975  | 0.2  | 0.4 | 0.4 | 0.2 | 0.2 | 0.5 | 0.3 | 0.7 | 0.2 | 0.8 | 0.7 | 0.5 | 0.5 | 0.9 | 0.5 | 0.2 |
| α-Phellandrene | 995 | 1003 | t    | t   | t   | t   | t   | t   | t   | 0.1 | t  | t  | t  | t  | t  | t  | t  | t  |
| δ-3-Carene | 1000 | 1004 | t    | t   | t   | t   | t   | t   | t   | t   | t  | t  | t  | t  | t  | t  | t  | t  |
| α-Terpinine| 1002 | 1005 | 0.1  | 0.1 | 0.1 | 0.4 | t  | 0.1 | 0.3 | 0.2 | 0.3 | 0.3 | 0.3 | t  | t  | t  | t  | 0.4 | 0.3 | t  |
| p-Cymene (1)| 1003 | 1008 | 12.3 | 16.2 | 9.7 | 24.4 | 12.2 | 9.9 | 8.8 | 35.0 | 11.7 | 19.4 | 19.1 | 23.4 | 12.9 | 13.8 | 9.3 | 12.4 |
| β-Phellandrene | 1005 | 1009 | 0.2  | 0.6 | 0.3 | 0.4 | 0.2 | 0.4 | 0.4 | 0.4 | 0.2 | 0.3 | 0.2 | 0.3 | 0.2 | 0.2 | 0.2 | 0.2 |
| Limonene (2) | 1009 | 1016 | 9.8  | 17.0 | 13.5 | 15.6 | 9.2 | 17.6 | 14.8 | 25.8 | 8.3 | 19.1 | 17.2 | 14.4 | 13.8 | 21.3 | 15.1 | 9.6 |
| γ-Terpinene (3)| 1035 | 1035 | 4.4  | 9.2 | 9.3 | 3.0 | 0.2 | 9.1 | 4.4 | 1.7 | 3.5 | 11.6 | 8.1 | 6.3 | 10.9 | 15.7 | 9.7 | 5.7 |
| trans-Sabinene hydrate | 1037 | 1047 | t    | t   | t   | t   | t   | t   | t   | t   | t  | t  | t  | t  | t  | t  | t  | t  |
| Terpinolene | 1064 | 1064 | t    | 0.1 | 0.2 | 0.1 | 0.1 | 0.2 | 0.2 | t  | 0.1 | 0.1 | t  | 0.1 | 0.2 | 0.2 | 0.2 | 0.1 |
| cis-Sabinene hydrate | 1066 | 1081 | 0.1  | 0.1 | 0.1 | t   | t   | 0.2 | 0.1 | t  | 0.1 | t  | 0.1 | 0.1 | 0.1 | 0.1 | 0.2 | 0.1 |
| Linalool | 1074 | 1082 | t    | t   | t   | t   | t   | 0.1 | 0.1 | t  | 0.1 | t  | t  | 0.1 | 0.1 | 0.1 | 0.1 | t  |
| trans-p-2-Menth-1-ol | 1099 | 1095 | 0.1  | 0.1 | 0.2 | t   | t   | 0.3 | 0.6 | t  | 0.1 | 0.1 | t  | 0.1 | 0.2 | 0.3 | 0.2 | t  |
| cis-p-2-Menth-1-ol | 1110 |      | t    | 0.1 | 0.1 | t   | t   | 0.1 | 0.3 | t  | t  | t  | t  | 0.1 | 0.1 | 0.2 | t  | t  |
| Terpinen-4-ol | 1148 | 1153 | 1.5  | 1.0 | 1.0 | 0.7 | 1.7 | 1.3 | 3.6 | 0.2 | 1.6 | 0.6 | 0.7 | 0.8 | 1.2 | 1.0 | 1.7 | 1.1 |
| α-Terpinol | 1159 | 1157 | 0.1  | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.6 | t  | 0.2 | 0.1 | 0.1 | 0.1 | 0.2 | 0.2 | 0.3 | 0.2 |
| Cumin aldehyde | 1200 | 1200 | t    | t   | t   | t   | t   | t   | t   | t   | t  | t  | t  | t  | t  | t  | t  | t  |
| Carvone | 1210 | 1206 | t    | t   | t   | t   | t   | t   | 0.1 | t  | t  | t  | t  | t  | t  | t  | t  | t  |
| Methyl thymol | 1210 |      | t    | t   | t   | t   | t   | t   | 0.2 | t  | 0.1 | t  | 0.1 | 0.1 | t  | t  | t  | t  |
| Cumin alcohol (4) | 1260 | 1270 | 30.6 | 24.3 | 13.4 | 48.6 | 15.4 | 5.1 | 2.1 | 10.6 | 36.6 | 14.4 | 10.3 | 46.9 | 12.0 | 4.3 | 2.0 | 19.4 |
| Thymol (5) | 1275 | 1278 | 39.8 | 28.9 | 49.8 | 4.0 | 59.6 | 53.0 | 60.0 | 22.8 | 36.3 | 31.2 | 42.1 | 5.0 | 45.5 | 39.3 | 57.7 | 49.1 |
| Carvacrol | 1286 | 1299 | 0.3  | 0.2 | 0.4 | 0.3 | 0.3 | 0.5 | 0.5 | 0.6 | 0.3 | 0.2 | 0.3 | 0.3 | 0.3 | 0.5 | 0.5 | 0.4 |

% of identification: 99.8 99.4 99.8 99.3 99.8 99.3 97.5 99.2 99.7 99.9 99.6 99.5 99.2 99.2 98.7 99.0

Grouped components:
- Monoterpene hydrocarbons: 27.3 44.5 34.6 45.5 22.4 38.4 29.2 65.0 24.3 53.3 46.1 46.1 39.6 53.4 35.7 28.5
- Oxygen-containing monoterpenes: 72.5 54.9 65.2 53.8 77.4 60.9 68.3 34.2 75.4 46.6 53.5 53.4 59.6 45.8 63.0 70.5

Yield (% v/w): 2.1 1.1 1.5 1.7 1.8 1.4 3.0 2.2 2.6 3.5 2.4 2.8 2.7 2.4 3.6 2.6

RI – In lab calculated Retention index relative to C<sub>9</sub>-C<sub>23</sub> n-alkanes on the DB-1column; RI* - reported literature retention indices on DB-1 or similar phase column (100% Dimethylpolysiloxane) not from the authors lab [24]. t - trace (<0.05%). * SS: Sidi Safi; T: Terni; O: Ouzidane; BS: Beni Snous; AF: Ain Fezza; F: Fehoul; BA: Bordj Arima; AK: Ain Kihal
contrast, flower EO from Ain Kihal had higher percentage of thymol (49%) than the respective leaf EO (23%). It is noteworthy to refer that flower EO from Ain Fezza had much more γ-terpinene (11%) (Fig. 1) than leaf EO (<0.5%); and flower EO from Ain Kihal had lower amounts of p-cymene (12%) and limonene (10%) than the respective leaf EO (35% and 26%, respectively) (Table 2).

In both leaf and flower EOs from Beni Snous, cumin alcohol dominated, which may reinforce the existence of a possible new EO-type, already described [1] for one sample collected in Tlemcen. Other main components have been reported in A. pusilla EOs such as carvacrol, γ-terpinene, p-cymene, carvone, thymol, isothymol, depending on the geographical origin and the developmental stage [1 and references therein]. According to Russo et al. [16], p-cymene can be the intermediate for the hydroxylation step to thymol, carvacrol, p-cymene-8-ol and cuminyl alcohol.

### 3.2 Antioxidant activity

The capacity for scavenging free radicals as well as for preventing lipid peroxidation of A. pusilla leaves and flowers EOs of was evaluated (Table 3). Beni Snous, Sidi Safi, and Ain Kihal leaf EOs possessed the best capacity for scavenging the free DPPH (IC$_{50}$ = 79 µg/mL; IC$_{50}$ = 73 µg/mL; and IC$_{50}$ = 121 µg/mL, respectively), ABTS (IC$_{50}$ = 2 µg/mL; IC$_{50}$ = 3 µg/mL; and IC$_{50}$ = 2 µg/mL, respectively) and hydroxyl (IC$_{50}$ = 1 µg/mL; IC$_{50}$ = 2 µg/mL; and IC$_{50}$ = 2 µg/mL, respectively) radicals. Samples from Fehoul and Bordj Arima were the worst scavengers of those free radicals (Table 3). Concerning flower EOs, only one sample (Beni Snous) had the best capacity for scavenging the free radicals reported above (IC$_{50}$ = 76 µg/mL; IC$_{50}$ = 2 µg/mL and IC$_{50}$ = 1 µg/mL, respectively). The worst scavengers of free DPPH and ABTS radicals were those from Ain Kihal and Bordj Arima, whereas the worst scavengers of hydroxyl radicals were those from Sidi Safi and Ouzidane (Table 3). The capacity for scavenging DPPH and ABTS free radicals as well as for preventing lipid peroxidation of some samples is within the range found for BHT, generally used as control, according to the IC$_{50}$ values (89, 4 and 96 µg/mL, respectively) previously reported [17] and obtained in the same laboratory. Concerning the capacity for scavenging hydroxyl radicals, even the sample with the best activity showed worse ability than mannitol in which the IC$_{50}$ value found by us, and already published, was 0.7 µg/mL [17].

The best flower EOs for preventing lipid peroxidation were those obtained from plants from Terni and Ain Fezza. The leaf EOs from Terni were the best antioxidant (Table 3). The results showed that the most adequate samples for scavenging free radicals were not necessarily the same for preventing lipid peroxidation (Table 3).

The antioxidant activity of A. pusilla EO was already reported [1], that research considered that the best activity of these EOs could be attributed to a possible effect of both thymol and cumin alcohol, since these compounds were already reported as being a weak to moderate

### Table 3: Antioxidant activity of essential oils isolated from flowers and leaves of A. pusilla collected at different places of Algeria.

| Samples | Flowers          | Leaves           |
|---------|------------------|------------------|
|         | DPPH IC$_{50}$ µg/mL ± STE | ABTS IC$_{50}$ µg/mL ± STE | TBARS IC$_{50}$ µg/mL ± STE | Hydroxyl IC$_{50}$ µg/mL ± STE | DPPH IC$_{50}$ µg/mL ± STE | ABTS IC$_{50}$ µg/mL ± STE | TBARS IC$_{50}$ µg/mL ± STE | Hydroxyl IC$_{50}$ µg/mL ± STE |
| SS      | 106±10           | 3±0             | 71±6            | 35±4 ‡‡‡            | 73±18            | 3±0             | 65±10           | 2±5 ‡‡‡            |
| T       | 132±11           | 5±0             | 30±6            | 19±4 ‡             | 79±18            | 4±0             | 23±10           | 18±5             |
| O       | 154±11           | 4±0             | 38±6 ‡‡         | 36±4 ‡‡            | 123±18           | 4±0             | 55±10           | 6±5 ‡‡         |
| BS      | 76±11            | 2±0             | 36±6            | 1±4               | 79±18            | 2±0             | 92±10           | 1±5             |
| AF      | 141±11           | 5±0             | 29±6            | 1±4 ‡              | nd               | nd              | nd              | nd              |
| F       | 200±11           | 5±0             | 105±6           | 25±4 ‡‡‡‡          | 178±18           | 5±0             | 85±10           | 7±5 ‡‡‡‡        |
| BA      | 380±11           | 20±0 ‡‡‡‡       | 46±6 ‡‡‡‡       | 23±4 ‡‡‡‡          | 338±18           | 7±0 ‡‡‡‡       | 51±10           | 6±5 ‡‡‡‡        |
| AK      | 263±11 ‡‡‡‡      | 7±0 ‡‡‡‡       | 32±6 ‡‡‡‡       | 21±4 ‡‡‡‡          | 121±18 ‡‡‡‡      | 2±0 ‡‡‡‡       | 67±10 ‡‡‡‡      | 2±5 ‡‡‡‡        |

STE, standard error. nd: Not Determined. Values in the same column followed by the same letter are not significant different by the Tuckey’s multiple range test (p > 0.05). † SS: Sidi Safi; T: Terni; O: Ouzidane; BS: Beni Snous; AF: Ain Fezza; F: Fehoul; BA: Bordj Arima; AK: Ain Kihal.

*statistically significant differences between antioxidant activity in flower and leaf EOs (p≤0.05; Student t-test), **statistically significant differences between antioxidant activity in flower and leaf EOs (p≤0.01; Student t-test), ***statistically significant differences between antioxidant activity in flower and leaf EOs (p≤0.001; Student t-test).
antioxidant [18,19]. In the present work, a correlation among the major components (>5%) such as p-cymene, limonene, γ-terpinene, cumin alcohol and thymol, and the antioxidant activity (capacity for scavenging DPPH, ABTS and hydroxyl radicals as well as the capacity for preventing lipid peroxidation) was made (Table 4). The results showed a strong (p<0.01) negative correlation between IC₅₀ values of ABTS, DPPH, and hydroxyl scavenging activity and the percentages of p-cymene (r = -0.709, r = -0.690 and r = -0.650, respectively) and cumin alcohol (r = -0.741, r = -0.902 and r = -0.811, respectively) present in leaf EOs. These results showed that the capacity for scavenging those radicals was highly dependent on the percentages of p-cymene and cumin alcohol, in which higher levels of these monoterpenes were responsible for the best activities found. Positive correlations between thymol percentages and IC₅₀ values of ABTS (r = 0.877), DPPH (r = 0.752) and hydroxyl (r = 0.771) scavenging activities were obtained, which may mean that such aromatic monoterpenoid acts negatively as scavengers of those radicals.

For thymol, such results are unexpected because several works have demonstrated the capacity of this phenolic terpene for scavenging free radicals. Due to the relative high number of works demonstrating such ability of thymol for scavenging free radicals, only two references we cite here as examples, not only for their recent publication, but also for the possible mechanisms involved in those activities presented by the authors [20,21]. However, another recent publication demonstrated that several components, including thymol when at relative high concentrations acts as pro-oxidant [22]. In addition, the authors concluded that mixtures of thymol and carvacrol used during the experiment are also highly dependent on the proportions of these phenolic compounds in the mixture. In our opinion, the positive correlations between IC₅₀ values and percentages of thymol present in A. pusilla EOs may mean that the percentages of cumin alcohol in these EOs are within the range of antioxidant activity which superpose of antioxidant activity of thymol. The strong negative correlation (p<0.01) between IC₅₀ values of ABTS (r = -0.634) and DPPH (r = -0.720) and cumin alcohol percentages were also observed for flower EOs, as well as the positive correlations (r = 0.844 and r = 0.806, for ABTS and DPPH, respectively) between IC₅₀ values and the percentages of thymol (Table 4). These results revealed, therefore, the possible antagonism and/or synergism effects among EOs constituents, indicating that caution should be taken on the use of the EOs as natural antioxidants due to the possible unexpected activities.

A negative correlation was also observed between IC₅₀ values of ABTS and DPPH methods and the percentages of p-cymene in the flower EOs (r = -0.576 and r = -0.550, respectively; p<0.05) (Table 4). No correlation between IC₅₀ values of hydroxyl scavenging activity and percentage was found. These results showed the importance of p-cymene on the capacity of this aromatic terpene on the capacity for scavenging free radicals. The antioxidant activity of p-cymene was also very recently reported [23].

No correlation was observed between the percentages of limonene and the IC₅₀ values found for every assay or between IC₅₀ values for TBARS assay and the EOs major components.

Ferric ion reduction can be used as an indicator of electron-donating activity and therefore reflects an important mechanism of antioxidant action. In this study, the reducing power was evaluated by monitoring the ferric-ferrous transformation at 700 nm. All flower EOs samples presented the capacity for reducing ferric to ferrous ion, although the sample with the best activity was that from AF (Ain Fezza) immediately followed by that from SS (Sidi Saïf), in contrast to that from BA (Bordj Arima), which presented the worst ability for reducing ferric ion. The activity of all samples was dose-dependent (Fig. 2). The worst activity of leaf EO was also from BA sample, whereas

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Table 4: Spearman correlation coefficients among compounds and antioxidant activity.

|            | Leaves ABTS | Leaves DPPH | Leaves Hydroxyl | Flowers ABTS | Flowers DPPH | Flowers Hydroxyl |
|------------|-------------|-------------|-----------------|-------------|-------------|-----------------|
| p-Cymene   | -0.709**    | -0.690**    | -0.650**        | -0.576*     | -0.550*     |                |
| Limonene   | -           | -           | -               | -           | -           | -               |
| γ-Terpinene| 0.596*      | -           | 0.587*          | -           | -           | -               |
| Cumin alcohol | -0.741**    | -0.902**    | -0.811**        | -0.634**    | -0.720**    | -               |
| Thymol     | 0.877**     | 0.752**     | 0.771**         | 0.844**     | 0.806**     | -               |

-- Not significant. * Correlation is significant at the p<0.05 level. ** Correlation is significant at the p<0.01 level.
the best activities were found in those samples from BS (Beni Snous) and T (Terni), with a similar activity (Fig. 3).

A single electron transfer-based reaction is the mechanism involved in the reduction of ferric to ferrous ion as in those methods of scavenging ABTS and DPPH free radicals [1]. So, the worst activity of both flower and leaf EOs from Bordj Arima are coincident with the worst scavengers of DPPH and ABTS free radicals, though not always the best EOs for reducing ferric ion had been those with the best capacity for scavenging free radicals.

Overall, significant differences of activities were found in A. pusilla EOs depending on the harvesting places. Differences were also detected according to the plant part used (flower or leaf). Significant differences of antioxidant activities between leaf and flower EOs from Ain Kihal were observed whatever the method used (Table 3). With the exception of TBARS method, the leaf EOs had higher ability for scavenging DPPH, ABTS and hydroxyl radicals (p<0.001) than flower EOs. Generally the capacity for scavenging free radicals was higher in leaf EOs. More examples include the capacity for scavenging DPPH radicals which were better in leaf EO from Terni and Ouzidane (p<0.05); or the capacity for scavenging ABTS radicals that was also higher in leaf EO of Bordj Arima (p<0.001). The capacity for scavenging hydroxyl radicals were also significantly higher (p<0.01) in leaf EOs from Sidi Safi and Ouzidane (Table 3).

3 Conclusion

The chemical compositions as well as the antioxidant activities of A. pusilla EOs, measured through different methods, were dependent on the harvesting place of the samples and plant part used. In many cases, leaf EOs had best capacity for scavenging free radicals or preventing lipid peroxidation than flower EOs. A negative correlation between IC<sub>50</sub> values of DPPH and ABTS free radicals and p-cymene and cumin alcohol percentages was found, which reveal that both compounds are positively involved on the scavenging of these radicals, although the role of the remaining constituents of the EOs on such activities may be also determinant, and should not be forgotten. Nevertheless, the results indicated that p-cymene and cumin alcohol were determinant on the ability of A. pusilla EOs for scavenging free radicals.

Conflicts of interest: The authors declare no conflicts of interest.

Figure 2: Reducing power of flower essential oils from Ammoides pusilla collected in diverse regions of Algeria. For collection places code, see Table 1.

Figure 3: Reducing power of leaf essential oils from Ammoides pusilla collected in diverse regions of Algeria. For collection places code, see Table 1.

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References

[1] Tefiani C., Riazi A., Youcefi F., Aazza S., Gago C., Faleiro ML, Pedro L.G., Barrosa J.G., Figueiredo A.C., Megías C., Cortés-Giraldo I., Vioque J., Miguel, M.G., Ammoides pusilla (Apiaceae) and Thymus munbyanus (Lamiaceae) from Algeria essential oils: chemical composition, antimicrobial, antioxidant and antiproliferative activities, J. Essent. Oil Res., 2015, 27, 131-139.

[2] Laouer H., Zerroug M.M., Sahli F., Chaker A.N., Valentini G., Ferretti G., Grande M., Anaya J., Composition and antimicrobial
activity of *Ammoides pusilla* (Brot) Breistr. essential oil, J. Essent. Oil Res., 2003, 15, 135-138.

[3] El Ouariachi E.M., Tomi P., Bouyanzer A., Hammouti B., Desjobert J.-M., Costa J., Paolini J., Chemical composition and antioxidant activity of essential oils and solvent extracts of *Phytchotis verticillata* from Morocco, Food Chem. Toxicol., 2011, 49, 533–536.

[4] Laouer H., Zerroug M.M., Chaker A.N., Bouzerzour H., Study of the effect of *Ammoides pusilla* (Brot.) Breistr, essential oil against *Pseudomonas* sp., Commun. Agric. Appl. Biol. Sci., 2004, 69, 619-624.

[5] Zerroug M.M., Laouer H., Strange R.N., Nicklin J., The effect of essential oil of *Ammoides pusilla* (brot.) Breistr on the growth and the production of solanapyrone A by *Ascochyta rabiei*, Comm. Agric. Appl. Biol. Sci., 2010, 75, 721-724.

[6] Oumessaad T., Abdelghani D., Cherifa H., Phytochemical study and antimicrobial activity of *Ammoides verticillata*, an Algerian endemic species, Curr. Opin. Biotechnol., 2011, 22, 5143-5143.

[7] Bouizam M., Merhfour F.Z., Legssyer A., Mekhfli H., Maâlem S., Ziyyat A., Antihyperglycemic activity of *Artabotrus unedo*, *Ammoides pusilla* and *Thymelaea hirsute*, Pharmazie, 2007, 62, 630-632.

[8] Bouizam M., Merhfour F.Z., Ziyyat A., Aziz M., Legssyer A., Mekhfli H., Antidiabetic effect of some medicinal plants of Oriental Morocco in neonatal non-insulin-dependent diabetes mellitus rats, Hum. Exp. Toxicol., 2010, 29, 865-871.

[9] Figueiredo A.C., Barroso J.G., Pedro L.G., Scheffer J.C., Factors affecting secondary metabolite production in plants: volatile components and essential oils, Flavour Fragr. J., 2008, 23, 213-226.

[10] Miguel M.G., Antioxidant and anti-inflammatory activities of essential oils: a short review, Molecules, 2010, 15, 9252-9287.

[11] Council of Europe (COE) *European Directorate for the Quality of Medicines*. European Pharmacopoeia, 6th edn. COE: Strasbourg (2007).

[12] Dandlen S.A., Lima A.S., Mendes M.D., Miguel M.G., Faleiro M.L., Sousa M.J., Pedro L.G., Barroso J.G., Figueiredo A.C., Antioxidant activity of six Portuguese thyme species essential oils, Flavour Fragr. J., 2010, 25, 150-155.

[13] Belhattach R., Amor L., Barroso J.G., Pedro L.G., Figueiredo A.C., Essential oil from *Artemisia herba-alba* Asso grown wild in Algeria: variability assessment and comparison with an updated literature survey, Arabian J. Chem., 2014, 7, 243–251.

[14] Aazza S., Lyoussi B., Miguel M.G., Antioxidant activity of some Moroccan hydrosols, J. Med. Plants Res., 2011, 5, 6688–6696.

[15] Aazza S., Lyoussi B., Miguel M.G., Antioxidant and antiacetylcholinesterase activities of some commercial essential oils and their major compounds, Molecules, 2011, 16, 7672-7690.

[16] Boulanouar B., Abdelaziz G., Aazza S., Gago C., Miguel M.G., Antioxidant activities of eight Algerian plant extracts and two Essentials oils, Ind. Crops Prod., 2013, 46, 85-96.

[17] Russo M., Galletti G.C., Bocchini P., Carnacini A., Essential oil composition of wild populations of Italian oregano spice (*Origanum vulgare* ssp. hirtum (Link)jetswaert): a preliminary evaluation of their use in chemotaxonomy by cluster analysis. 1. Inflorescences. J. Agric. Food Chem., 1998, 46, 3741-3746.

[18] Teissedre P.L., Waterhouse, A.L., Inhibition of oxidation of human low-density lipoproteins by phenolic substances in different essential oils varieties, J. Agric. Food Chem., 2000, 48, 3801-3805.

[19] Lee S.-J., Umano K., Shibamoto T., Lee K.-G., Identification of volatile compounds in basil (*Ocimum basilicum* L.) and thyme leaves (Thymus vulgaris L.) and their antioxidant properties, Food Chem., 2005, 91, 131-137.

[20] Beena Kumar, D., Rawat D.S., Synthesis and antioxidant activity of thymol and carvacrol based Schiff bases, Bioorg. Med. Chem. Lett., 2013, 23, 641-645.

[21] Sharopov F.S., Wink M., Selzer W.N., Radical scavenging and antioxidant activities of essential oils components – an experimental and computational investigation, Nat. Prod. Commun., 2015, 10, 153-156.

[22] LLiana-Ruiz-Cabello M., Gutiérrez-Praena D., Puerto M., Richardo S., Jos A., Caneán A.M., *In vitro* pro-oxidant/antioxidant role of carvacrol, thymol and their mixture in the industrial Caco-2 cell line, Toxicol. Vitro, 2015, 29, 647-656.

[23] Oliveira T.M., Carvalho R.B.F, Costa I.H.F., Oliveira G.A.L., Souza A.A., Lima S.G., Freitas R.M., Evaluation of *p*-cymene, a natural antioxidant, Pharm. Biol., 2015, 53, 423-428.

[24] Linstrom, P.J., Mallard, W. G. Eds., *NIST Chemistry WebBook*, NIST Standard Reference Database Number 69, National Institute of Standards and Technology, Gaithersburg MD, 20899, http://webbook.nist.gov.