IMPACT OF GEOGRAPHIC’S VARIATION ON THE ESSENTIAL OIL YIELD AND CHEMICAL COMPOSITION OF THREE Eucalyptus SPECIES ACCLIMATED IN TUNISIA

Elaissi Ameur¹*, Medini Hanene¹, Rouis Zied², Khouja Mohamed Larbi³, Chemli Rachid¹ and Harzallah-Skhiri Fethia¹

¹Laboratory of The Chemical, Galenic and pharmacological Drug Development, Faculty of Pharmacy, University of Monastir, Avenue Avicenne, 5019 Monastir, Tunisia
²Laboratory of Genetic, Biodiversity and Bio-resources Valorisation, Higher Institute of Biotechnology of Monastir, University of Monastir, Avenue Tahar Haddad, 5000 Monastir, Tunisia
³National Institute for Research on Rural Engineering, Water and Forestry, Institution of Agricultural Research and Higher Education, BP. N.2, 2080 Ariana, Tunisia

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ABSTRACT

Present study has been carried out to estimate the impact of geographical distribution on the yield and chemical constitute of three Eucalyptus verities viz E. cinerea F. Muell. ex Benth., E. astringens Maiden and E. sideroxylon A.Cunn. ex Schauer-. These species were collected from six arboreta of Tunisia in January 2008. The essential oil was extracted by hydrodistillation method and estimated the essential oil yield which varies from 1.5±0.1% to 4.0±0.2%. Results of the study revealed that yield of essential oil are not only depends on the Eucalyptus species but also depends on the origin of harvest. E. sideroxylon A. Cunn. exWoolls, cultivated in jbel abderrahman arboreta and E. cinerea F. Muell. ex Benth. from choucha (sejnanae) arboreta provided the lowest and the highest percentage of essential oil amongst all the studied provenances, respectively. GC (RI) and GC/MS analysis showed the presence of 163 components, representing 98.8 to 99.5% of the total oil. The contents of the different samples varied according to the species and the origin of harvest. The main components of the Eucalyptus essential oil were 1,8-cineole (39.1±0.0 – 79.4±0.0%), followed by α-pinene (2.1±0.0- 30.0±0.0), trans-pinocaveol...
1 Introduction

Genus *Eucalyptus* comprises about 900 species and subspecies (Pereira et al., 2014). More than 300 species of this genus contain volatile oil in their leaves. In 1957, total 117 *Eucalyptus* have been introduced in Tunisia. They were used essentially as fire wood, for the production of mine wood and against the erosion (Khouja et al., 2001). However, less than 20 species have ever been exploited commercially for the production of essential oil rich in 1,8-cineole (>70%) which is essentially used in the pharmaceutical and cosmetic industries (Pino et al., 2002). In Tunisian folk medicine, inhalation of *Eucalyptus* sp. essential oil has been traditionally used to treat respiratory tract disorders such as pharyngitis, bronchitis and sinusitis (Boukef, 1986). Many studies have been demonstrated their antibacterial, antifungal and antiviral activities of *Eucalyptus* sp. essential oil against a wide range of microorganisms (Su et al., 2006; Cermelli et al., 2008; Gilles et al., 2010; Iha et al., 2014). Furthermore, allelopathic effect of this essential oil against many weeds was also reported by many researchers (Batish et al., 2004; Verdeguer et al., 2009; Rassaeifar et al., 2013). *Eucalyptus* essential oil was also reported as an effective anti-inflammatory, analgesic, antioxidant, antimutagenic, insecticide, nematocide and acaricide oil (Batish et al., 2008; Bugarin et al., 2014; Rossi & Palacios, 2015). The *Eucalyptus* essential oils can be obtained by different methods, such as hydrodistillation, supercritical CO2 extraction, microwave-assisted extraction, and vacuum extraction by solvents. These methods affect the final yield of the oil and it varied from 0.1± 0.1 to 5.7±0.5 7% (Elaissi et al., 2010a; Elaissi et al., 2010b). High levels of 1,8-cineole (87.8%) were found for the majority of the *Eucalyptus* species (Zira et al., 2004; Elaissi et al., 2010c). Generally, the yields and composition of the oils varies and it depending on species, used part, plant origin zone, collection period, growth stage of the plant as well extraction methods and storage conditions (Arango-Bedoya et al., 2012).

In previous studies published on *Eucalyptus* species growing in Tunisia, characterization of the leaves essential oils of 48 *Eucalyptus* species and their antibacterial activities were carried out by the same authors. On the basis of this work three *Eucalyptus* species, *E. cinerea* F. Muell. ex Benth., *E. astringens* Maiden and *E. sideroxylon* A. Cunn. ex Schauer, which demonstrated the best antibacterial activity and also the best content in essential oil yield and 1,8-cineole, were select for this research work to identify the effect of the origin of harvest on their essential oils yield and composition.

2 Materials and Methods

2.1 Plant Materials

Clean and mature leaves of three *Eucalyptus* species L. Hér., *viz.* *E. astringens* Maiden, *E. cinerea* F. Muell. ex Benth. and *E. sideroxylon* A. Cunn. ex Schauer, were collected from six arboreta belonging to lower humid, higher humid and sub-humid bioclimatic stage of Tunisia in January 2008, Table 1. The leaves were dried in airy premises, shielded from the light, then packed in paper bags, and kept in the shade. Botanical voucher specimens of the collected species have been deposited in the Herbarium of the Pharmacognosy Laboratory, Faculty of Pharmacy, Monastir, Tunisia (references 0156, 0157, 0158, 0159, 0160, 0161, 0162, 0163, 0164, 0165, 0166 and 0167).

2.2 Sample preparation and extraction of Essential Oils

The essential oils were extracted by hydrodistillation method, for this 100 g of boorishly crushed *Eucalyptus* leaves for 4 h, using a standard apparatus recommended in the European Pharmacopoeia. Hydrodistillations were performed in triplicate for each *Eucalyptus* species. The oil collected from each plant was dried (Na2SO4) and stored at 4°C until analysis. The yield of essential oil was expressed in % (v/w) of the dry material (Elaissi et al., 2010a; Elaissi et al., 2010b).

2.3 Chemical analysis

2.3.1 GC Analysis

Quantitative and qualitative data of the essential oils were determined in triplicate by GC and GC/MS, respectively. GC Analysis was carried out with a Hewlett-Packard 6890 apparatus equipped with FID and a polar Carbowax column (30 m_0.32 mm i.d., film thickness 0.25mm). The oven temperature was programmed isothermal at 35°C for 1 min, rising from 35 to 250°C at 5°C/min, and then held isothermal at 250°C for 3 min; injector temp., 250°C; detector temp., 280°C; N2 used as carrier gas (1.2 ml/min).
| Eucalyptus species | Provenance (Arboreta) | Latitude | Longitude | Altitude (m) | Annual rainfall (mm) | Bioclimatique stage | Soil type | Abbreviation | Yield [%] |
|-------------------|----------------------|----------|-----------|--------------|---------------------|---------------------|-----------|--------------|----------|
| E. cinerea        | Souiniat              | 35°54'   | 8°48'     | 492          | 1140                | humid inferior with temperate winter | sandstone hydromorphe | cin Soui | 3.7±0.7 (a,b) |
| E. cinerea        | Zerniza               | 30°94'   | 9°7'      | 60           | 920                 | humid inferior with warm winter       | sandstone hydromorphe | cin Zer  | 3.8±0.4 (a)   |
| E. cinerea        | Sejnane               | 37°3'    | 9°14'     | 159          | 871                 | humid inferior with temperate winter | sandstone            | cin Sej  | 4.0±0.2 (c)   |
| E. astringens     | Mrifek                | 37°07'   | 9°15'     | 60           | 950                 | humid inferior with mild winter       | Marl                 | ast Mri  | 3.1±1.0 (c)   |
| E. astringens     | Korbous (Sejnane)     | 36°50'   | 10°35'    | 180          | 540                 | sub-humid with warm winter            | Sandy               | ast Kor  | 3.2±0.1 (c)   |
| E. astringens     | Pryor Choucha         | 37°3'    | 9°14'     | 159          | 871                 | humid inferior with temperate winter | sandstone            | ast Sej  | 3.3±0.3 (c,a) |
| E. sideroxylon    | Mrifek                | 37°07'   | 9°15'     | 60           | 950                 | humid inferior with mild winter       | Marl                 | sid Mri  | 2.6±0.3 (c)   |
| E. sideroxylon    | Jbel Abderrahmane     | 36°40'   | 10°40'    | 255          | 600                 | Sub-humid with warm winter            | sandy clay           | sid JBA  | 1.5±0.1 (c)   |
| E. sideroxylon    | Korbous               | 36°50'   | 10°35'    | 180          | 540                 | sub-humid with warm winter            | Sandy               | sid Kor  | 2.3±0.1 (c)   |
| E. sideroxylon    | Souiniat              | 35°54'   | 8°48'     | 492          | 1140                | humid inferior with temperate winter | sandstone hydromorphe | Sid Soui | 2.6±0.4 (c)   |

a) Yields with different letters in parentheses differ significantly by Duncan’s multiple range test (p<0.05).

The injected volume was 1 ml (10% essential oil in purified hexane). The relative concentration was calculated using the software HP Chemstation, which allows assimilating the percentages of the peak areas to the percentages of the various constituents. Retention indices were obtained by running a series of aliphatic hydrocarbons (C9 - C28) by increasing the number of carbon atoms in the Carbowax column (Elaissi et al., 2012) GC column).

2.3.2 GC/MS Analysis

The essential oils were analyzed with a Hewlett-Packard 5890 series II apparatus equipped with a 5972 mass-selective detector and polar Carbowax Column (30 m,0.32 mm i.d., 2.3.4 Statistical Analysis

The mass spectrometer operating conditions were: 70 eV ionization voltage, 70 eV; 230°C ion source. The GC analysis conditions were as described above (see GC Analysis) (Elaissi et al., 2012).

2.3.3 Compound Identification

Isolated compounds were based on the comparison of their RI (determined rel. to the tR of n-alkanes (C9–C28)) and mass spectra with those of authentic compounds by means of NBS75K.L. and Wiley 275 databases and with the literature data (Wiley & Sons, 1998).
The data were analyzed using analysis of variance (ANOVA), and the significance of the differences between means was determined at p<0.05 using Duncan’s multiple range test. To evaluate the identified essential oil constituents useful for chemical reaction between selected species, 16 compounds were identified from the oil samples with contents in the essential oils of 0.9% in at last one species, were subjected to PCA and HCA using SPSS 12.0 software (SPSS Inc. Chicago, IL, USA) (Elaissi et al., 2012).

3 Results and Discussion

3.1 Oil Yields

The Analysis of variance (ANOVA) indicated that the oil yields were significantly different between species to species and arboreta to arboreta (p<0.05). The analysis of results showed the presence of five overlapping groups (Table 1) ranging from 1.5±0.1% for E. sideroxylon cultivated in Jbel Abderrahmane arboreta to 4.0±0.2% for E. cinerea grown in Pyor Choucha (Sejname) arboreta (Table 1), which differed from the others arboreta (Souiniat and Zerniza) by a sandstone soil and by the lowest annual plymiometry. Results of the study also revealed that E. sideroxylon harvested from Souiniat and Mrifek arboreta, which were characterised by a higher annual raining, higher altitude and by a sandstone soil, produced a higher mean percentage of essential oils relatively to those collected from Jbel Abderrahman and Korbous arboreta, while E. astringens was not affected by the environmental conditions. These result was confirmed by Dunlop et al. (2000) who found that the essential oil yields of some clones of E. camaldulensis was not dependent on the sites at which they were grown but rather on their genetic constitution, however Bhatti et al. (2007) reported that E. cerebra essential oil yield varied significantly among different district of Punjab-Pakistan. The increasing level of essential yield was depend to environmental conditions such as dry, warm, altitude (Tsiri et al. 2003; Arango-Bedoya et al., 2012; Hassiotis et al., 2014). Furthermore Zrira et al. (1994) reported that E. sideroxylon from morocco, region of Jbilet was richer in essential oil (2.06%) than that of the region of Tekerkoust (1.36%), while E. astringens from Tekerkoust was the richest one (2.45%) against 2.20% for the other provenances. These results were also proved by Grbovic et al. (2010) which were found that the leaves essential oil yield of E. camaldulensis Dehn. collected from five regions of the coastline of Montenegro varied from 0.67% for those collected from Bar region to 1.59% for the region of Tivat. Similarly LI & Madden (1995) found that the essential oils obtained from leaves of E. regnans, E. deligatensis, E. nitens, E. denticulata and E. gobulus grown in eight arboreta varied significantly within species and within provenances. It was also demonstrated that E. cinerea from Morocco (Zrira et al., 2004) was poor in essential oils (0.26%) than those cultivated in India (2.55 to 2.87%) (Kiran Babu & Singh, 2009).

3.2 Chemical composition

The chromatographic analyses (GC (RI) and GC/MS) of the essential oils allowed the identification of 163 compounds (Table 2), representing 98.8 – 99.5% of the total oil. Their mean percentage varied within species and harvest zone. The identified components were divided in seven chemical classes (Table 2).The major class was constituted by the oxygenated monoterpenes (46.5±0.0 – 85.6±0.0), with 1,8-cineole (39.1±0.0– 79.4±0.0%) having the highest content in all the studies species, whatever their provenance. It was followed by α-pinene (0.2±0.0 – 8.8±1.1%) and α-terpinyl acetate (0.0 – 2.6±0.6%). The class with the second highest contents composed of the monoterpenes hydrocarbons (4.5±0.0 – 35.0±0.0%) represented by α-pinene (2.5±0.0 – 30.0±0.0%), limonene (1.0±0.0 – 4.3±0.8%), β-cymene (0.7±0.0 – 2.7±2.1%) and γ-terpinene (traces to 0.3±0.0%). The class with the third highest contents was composed by the oxygenated sesquiterpenes (1.4±0.4 – 12.8±0.0%), constituted essentially by globulol (0.3±0.0 – 5.7±1.3%), viridiflorol (0.2±0.0 – 1.8±0.0%), epiglobulol (traces to 1.3±0.0%) and spathulenol (0.4±0.0 – 1.3±0.0%). The sesquiterpenes hydrocarbons occupied the fourth position with a mean percentage varying between 1.4±0.1 and 8.0±2.1%, from wich the aromadendrene (0.1±0.0 - 4.9±2.1%) was the major compound, followed by bicyclogermacrene (traces to 3.1±0.0%), β-carophyllene (0.3±0.0 - 1.9±0.0%) and allo-aromadendrene (0.1±0.0 – 0.9±0.0%). The ketones, esters and others were minor compound classes, not discussed in further details. The comparative study of the essential oils chemical composition according to the species and their origin of harvest (arboreta), shows that E. sideroxylon oils obtained from leaves harvested from Korbous arboreta are richer in oxygenated monoterpenes (85.6±0.0%) and in 1,8-cineole (79.4±0.0), while it was it was characterized by the lowest content in α-pinene (2.1±0.0). E. cinerea from Souiniat arboreta was also characterised by a relatively high mean percentage of 1,8-cineole (71.8±0.0) against 69.8±2.2% and 70.7±1.5% for those from Souiniat and Zerniza arboreta; respectively, which were distinguished by their highest mean percentages of α-terpinol (8.8±1.1, 7.5±0.6%; respectively), limonene (4.3±0.8, 3.8±0.0%; respectively) and α-terpinyl acetate (2.6±0.6, 2.1±0.6% ; respectively). Oils of all the studied population of E. astringens oils have the lowest mean percentage in oxygenated monoterpenes (16.5±1.9 – 35.0±0.0%) and in 1,8-cineole (39.1±0.0 – 47.6±1.2%), however they were characterized by the highest mean percentage in α-pinene, tr-pinocarveol, pinocarvone and attend the maximum in that of Korbous, Mrifek and Sejname arboreta; respectively. It was also observed that all the provenance of E. astringens are relatively rich in sesquiterpenes hydrocarbons (6.8 ± 0.3 – 8.0 ± 2.1%), aromadendrene, allo-aromadendrene and bicyclogermacrene and in oxygenated sesquiterpenes (9.7±0.0 – 11.1±0.1%) represented essentially by globulol, epiglobulol and guaiol.
| Compounds and names | KI | Composition (%) |
|---------------------|----|-----------------|
|                     | E. cinerea | E. australis | E. sideroxylon |
|                     | Souiniat | Zerina | Pryor Choucha (Sejnane) | Miljek | Korbas | Pryor Choucha (Sejnane) | Miljek | Sejnane (Choucha) | Jbel Abderraham | Korbas | Souiniat |
| **Monoterpenes**    |     |     |     |     |     |     |     |     |     |     |
| Hydrocarbons        | 11.1 | 10.2 | 10.3 | 24.4 | 35 | 16.5 | 15.4 | 8.2 | 4.5 | 4.9 | 10.5 |
| Tricyclicene        | 1043 | - | tr | tr | - | - | - | - | Tr | - | - |
| α-Pinenene          | 1050 | 5.1 | 5.2 | 5.7 | 21.8 | 30.0 | 14 | 8.3 | 2.9 | 2.5 | 2.1 | 5.1 |
| α-Thyene            | 1053 | tr | tr | tr | tr | 0.1 | tr | 0.2 | 0.1 | - | tr | tr |
| α-Fenchene          | 1072 | tr | tr | tr | tr | 0.1 | tr | tr | tr | tr | tr |
| Camphene            | 1077 | 0.1 | 0.1 | 0.1 | 0.2 | 0.1 | 0.1 | 0.1 | 0.1 | tr | 0.1 | 0.1 |
| α-Pinenene          | 1108 | 0.1 | tr | 0.1 | 0.2 | 0.5 | 0.2 | 0.2 | 0.1 | 0.1 | 0.1 | 0.2 |
| Sabinene            | 1125 | - | - | - | - | - | tr | - | - | tr |
| Verbenene           | 1127 | - | - | - | 0.1 | tr | 0.1 | tr | - | - | tr | tr |
| 2,4(10)-Tujadiene   | 1132 | - | - | - | - | - | tr | - | - | - | - |
| σ-3-Carene          | 1146 | - | - | - | - | - | - | tr | - | - | - |
| α-Phellandrene      | 1168 | 0.1 | 0.1 | 0.1 | 0.1 | 0.4 | 0.1 | 1.3 | 0.2 | 0.1 | tr | 0.4 |
| Myrcene             | 1172 | tr | tr | tr | tr | - | - | tr | - | tr | tr |
| α-Terpine           | 1183 | - | - | - | - | - | - | 0.1 | - | - | - |
| Limonene            | 1207 | 4.3 | 3.8 | 3.3 | 1 | 1.9 | 0.6 | 3.9 | 1.7 | 1 | 1.5 | 3.3 |
| α-Phellandrene      | 1209 | - | - | - | - | - | - | - | - | - | - | 0.3 |
| α-cis-Ocimene       | 1240 | tr | tr | - | - | - | tr | tr | - | tr | tr |
| α-Terpine           | 1251 | 0.1 | 0.1 | tr | tr | tr | tr | 0.3 | 0.3 | tr | 0.1 | 0.1 |
| α-trans-Ocimene     | 1256 | tr | tr | tr | 0.1 | tr | tr | tr | 0.1 | 0.1 | 0.1 | tr |
| p-Cymene            | 1278 | 1 | 0.7 | 0.7 | 0.8 | 1.7 | 1.3 | 0.9 | 2.7 | 0.7 | 0.8 | 0.8 |
| Terpinolene         | 1289 | 0.1 | 0.1 | 0.1 | 0.1 | tr | 0.1 | tr | 0.1 | tr | - | 0.1 |
| **Oxgenated monoterpenes** | 85 | 84.9 | 85 | 56.4 | 46.5 | 64 | 75.1 | 77.8 | 70.4 | 85.6 | 80.2 |
| p-α-Dimethyl styrene | 1449 | 0.1 | 0.1 | 0.1 | tr | tr | tr | tr | tr | tr | tr |
| 1.8-Cineole         | 1218 | 69.8 | 70.7 | 71.8 | 40.1 | 39.1 | 47.6 | 66.7 | 71.2 | 64.1 | 79.4 | 72.3 |
| p-Mentha-6.8-dien-2-ol | 1222 | tr | tr | tr | tr | - | - | tr | tr | - | tr |
| trans-Rose oxyde    | 1366 | - | - | - | 0.1 | 0.1 | - | - | - | - | - |
| α-α-Pinene epoxide  | 1384 | tr | tr | - | - | - | tr | - | - | - | - |
| α-Thujone           | 1423 | 0.1 | 0.1 | 0.1 | tr | - | tr | tr | - | tr | tr |
| cis-Linalool oxyde  | 1455 | tr | - | - | - | - | - | - | - | - | tr |
| trans-Linalool oxyde| 1468 | tr | tr | tr | - | - | - | tr | - | - | - |
| Citronellal         | 1491 | - | - | - | - | - | - | tr | - | - | - |
| α-Campholenic aldehyde | 1502 | tr | tr | tr | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | tr | tr |
| Camphor             | 1531 | tr | tr | tr | - | - | - | tr | - | - | tr |
| iso-Pinocamphone    | 1533 | - | - | - | 0.1 | tr | 0.1 | - | - | - | - |
| Linalool            | 1560 | tr | - | - | tr | tr | tr | tr | - | tr | - |
| trans-p-Menth-2-en-1-ol | 1572 | tr | - | tr | - | - | tr | tr | - | - | tr |

Impact of Geographic’s variation on the essential oil yield and chemical composition of three Eucalyptus species acclimated in Tunisia.
| Compound                                      | Value  |
|----------------------------------------------|--------|
| cis-Sabinene hydrate                         | 1576   |
| Isopulegol                                   | 1581   |
| Pinocarvone                                  | 1585   |
| Fenchol                                      | 1593   |
| Terpinene-4-ol                               | 1618   |
| cis-p-Menth-2-en-1-ol                        | 1634   |
| Myrtenal                                     | 1638   |
| Umbellulone                                  | 1655   |
| trans-pinocarvone                            | 1670   |
| trans-p-Menth-2-ene-1.8-diol                 | 1673   |
| α-Terpineol                                  | 1686   |
| Cis-Piperitol                                | 1698   |
| CarvotanAcetone                              | 1699   |
| Nerol                                        | 1705   |
| α-Terpinyl acetate                          | 1708   |
| Borneol                                      | 1710   |
| Phellandral                                  | 1740   |
| Piperitone                                   | 1746   |
| Carvone                                      | 1754   |
| Geranial                                     | 1757   |
| Geranial                                     | 1761   |
| Geranyl acetate                              | 1763   |
| trans-Piperitol                              | 1767   |
| Citronellol                                  | 1785   |
| Cuminal                                      | 1801   |
| Cuminal                                      | 1803   |
| Myrtenol                                     | 1807   |
| trans-p-Menth-1(7).8dien-2-ol                | 1811   |
| Nerol                                        | 1818   |
| α-Phellandrene epoxide                       | 1822   |
| trans-Cardoeval                              | 1848   |
| trans-p-Menth-1.8-dien-6-ol                  | 1856   |
| p-Cymen-8-ol                                | 1863   |
| Geraniol                                     | 1867   |
| Neryl acetone                                | 1875   |
| cis-Carvone                                  | 1879   |
| cis-p-Menth-1.8-dien-6-ol                    | 1884   |
| cis-p-Menth-1(7).8dien-2-ol                  | 1903   |
| p-Menth-1.5-dien-7-0                         | 1984   |
| Methyl eugenol                               | 2026   |
| p-Cymen7-ol                                  | 2121   |
| Thymol                                       | 2172   |
| Eugenol                                      | 2191   |
| Carvacrol                                    | 2233   |
| Carvacrol                                    | 2239   |
Impact of Geographic’s variation on the essential oil yield and chemical composition of three *Eucalyptus* species acclimated in Tunisia.

| *p*-Cuminol    | 2240 | - | - | - | - | - | - | - | - |
| 1-Terpinol     | 1643 | - | - | tr | - | - | 0.1 | tr | tr | tr | tr | - |
| 1.8-Menthadien-4-ol | 1701 | 0.1 | 0.1 | 0.2 | - | - | 0.1 | 0.1 | 0.1 | 0.1 | tr | tr |

**Sesquiterpene hydrocarbons**

|          | 1.6 | 1.8 | 1.4 | 1.0 | 7.7 | 6.8 | 4.8 | 4.8 | 10.5 | 3.2 | 4.1 |
|----------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|
| Isolatedene    | 1477 | - | 0.1 | 0.1 | 0.2 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | tr | tr | 0.1 |
| α-Cubebeene    | 1479 | 0.1 | - | - | tr | - | - | tr | - | - | - | - |
| Bicycloelemene | 1493 | - | - | - | tr | tr | - | tr | - | - | - | - |
| α-Copaene      | 1506 | tr | - | - | - | - | - | - | - | - | - | - |
| α-Gurjuneene   | 1542 | tr | tr | tr | tr | tr | 0.1 | tr | 0.1 | 0.1 | tr | tr |
| β-Cubebeene    | 1554 | tr | tr | tr | tr | tr | - | tr | tr | - | tr | - |
| β-Elemene      | 1602 | - | - | - | tr | tr | - | tr | tr | - | tr | 0.1 |
| β-Gurjuneene   | 1607 | - | - | - | 0.2 | 0.1 | 0.2 | tr | 0.1 | 0.1 | 0.1 | 0.1 |
| β-Caryophyllene| 1613 | 0.7 | 0.6 | 0.6 | 0.3 | 1 | 1.4 | 0.8 | 1.9 | 0.4 | 1 |
| α-Guaiene      | 1619 | - | - | - | - | - | - | - | - | - | - | - |
| Aromadendrene  | 1622 | 0.2 | 0.2 | 0.1 | 4.9 | 3.8 | 3.8 | 0.4 | 1.8 | 3.2 | 1.2 | 0.5 |
| Dehydroramadendrene | 1630 | tr | tr | tr | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Alloaromadendrene | 1661 | 0.1 | 0.2 | 0.1 | 0.6 | 0.6 | 0.2 | 0.5 | 0.9 | 0.4 | 0.2 |
| α-Humulenene   | 1683 | 0.2 | 0.3 | 0.2 | 0.1 | tr | 0.1 | 0.2 | 0.2 | 0.2 | 0.2 | 0.3 |
| Ledene         | 1703 | tr | tr | tr | tr | tr | tr | tr | - | - | - | - |
| Germacrene D   | 1712 | - | - | - | - | - | - | - | - | - | - | - |
| β-Selinene     | 1715 | tr | - | - | 0.5 | 0.2 | 0.4 | - | - | - | - | - |
| α-Selinene     | 1727 | - | - | tr | tr | tr | 0.1 | tr | 0.1 | 0.1 | 0.1 | 0.1 |
| A-Murolene     | 1740 | tr | tr | tr | 0.4 | 0.2 | - | 0.2 | tr | - | tr | 0.3 |
| Bicyclogermacrene | 1754 | tr | - | - | 0.2 | 0.5 | 0.1 | 1.6 | 0.6 | 3.1 | 0.4 | 1.1 |
| α-Cadinene     | 1768 | tr | tr | tr | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| γ-Cadinene     | 1773 | tr | tr | - | tr | tr | tr | tr | - | tr | - | tr |
| cis-Calamenene | 1839 | tr | 0.1 | 0.1 | - | - | - | tr | tr | - | tr | - |
| α-Calacorene   | 1938 | - | - | - | - | - | - | - | tr | - | tr | - |
| Eremophiliene  | 2137 | 0.1 | 0.2 | 0.1 | 0.5 | 0.6 | 0.4 | 0.2 | 0.3 | 0.7 | 0.3 | 0.2 |
| β-Maaliene     | 2162 | - | - | - | - | tr | tr | tr | tr | - | tr | - |
| α-Amorphene    | 1690 | - | - | - | tr | - | - | - | - | - | - | - |

**Oxygenated Sesquiterpenes**

|                 | 1.6 | 2.2 | 1.4 | 1.0 | 9.7 | 11.1 | 3.6 | 7.6 | 12.8 | 5 | 4.1 |
|-----------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|
| Palustrol       | 1950 | tr | 0.1 | tr | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.2 | 0.1 | 0.1 |
| Caryophyllene oxyde | 2010 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.2 | 0.1 | 0.1 |
| Epiglobulol     | 2033 | 0.1 | tr | 1.3 | 0.9 | 1.1 | 0.1 | 0.5 | 0.7 | 0.3 | 0.1 |
| Ledol           | 2053 | tr | 0.1 | tr | 0.2 | 0.2 | 0.2 | 0.1 | 0.2 | 0.3 | 0.1 | 0.1 |
| trans-Nerolidol | 2068 | tr | - | - | tr | tr | 0.1 | tr | - | tr | tr | tr |
| Elemol          | 2075 | 0.1 | 0.2 | 0.1 | 0.4 | 0.4 | 0.3 | 0.2 | 0.3 | 0.6 | 0.2 | 0.2 |
| β-Oplopenone    | 2087 | - | - | - | tr | tr | tr | 0.1 | 0.1 | 0.1 | 0.1 |
| Globulol        | 2097 | 0.5 | 0.6 | 0.3 | 5.7 | 4.5 | 5.3 | 0.9 | 3 | 4.7 | 2.1 | 1 |
| Viridiflorol    | 2107 | 0.2 | 0.4 | 0.2 | 0.9 | 1 | 0.9 | 0.5 | 0.7 | 1.8 | 0.5 | 0.5 |
| Guaiol          | 2129 | 0.1 | 0.1 | 0.1 | 0.5 | 0.5 | 0.5 | 0.2 | 0.3 | 0.6 | 0.2 | 0.2 |
| Rosifoliot      | 2130 | 0.1 | tr | - | - | - | - | - | - | - | - | - |
| Spathuleno1     | 2145 | 0.2 | 0.3 | 0.2 | 0.4 | 1.1 | 1.1 | 0.5 | 0.8 | 1.3 | 0.5 | 0.6 |
| 7-Cadinol       | 2166 | - | - | - | tr | tr | 0.1 | 0.1 | tr | 0.2 | tr | 0.1 |
| Substance                  | Value |
|----------------------------|-------|
| T-Muurolool                | 2183  |
| γ-Eudesmol                 | 2190  |
| α-Cadinol                  | 2201  |
| Agarospirol                | 2208  |
| α-Cadinol                  | 2220  |
| β-Eudesmol                 | 2247  |
| Isopanthulenol             | 2253  |
| β-Eudesmol                 | 2258  |
| Farnesyl acetate           | 2271  |
| Isobicyclomeric            | 2336  |
| (E,E) Farnesol             | 2374  |
| Esters                     |       |
| Isobutyl isobutyrate       | 1091  |
| 4-Methyl-2-pentyl acetate  | 1109  |
| Isoamyl acetate            | 1126  |
| Isoamyl isovalerate        | 1300  |
| Hexyl isobutyrate          | 1334  |
| Isoamyl valerate           | 1410  |
| Isoamyl hexanoate          | 1466  |
| Amyl benzoate              | 1939  |
| Benzyl pentanoate          | 1956  |
| β-Phenylpropionate         | 2020  |
| Ketones                    |       |
| 6-Methyl-5-Hepten-2-one    | 1345  |
| Nopinone                   | 1591  |
| Cryptone                   | 1690  |
| 4-Methyl accepheneone      | 1795  |
| cis-Jasmine                | 1964  |
| Jackson                    | 2289  |
| Tasmanone                  | 2305  |
| Agglomerone                | 2399  |
| Lateriticone               | 2394  |
| Torquatone                 | 2432  |
| Others                     |       |
| Hexanal                    | 1095  |
| trans-2-Hexenal            | 1226  |
| Nonanal                    | 1401  |
| Diphenyl oxide             | 2004  |
| cis-3-Hexenol              | 1389  |
| 2-Nonanol                  | 1523  |
| O-cresol                   | 1648  |
| Toluene                    | 1063  |
| Total identified           | 99.5  |

The values are presented as percentages, where tr indicates the presence of the substance.
Impact of Geographic’s variation on the essential oil yield and chemical composition of three *Eucalyptus* species acclimated in Tunisia.

Figure 1 PCA of 24 compounds for the leaf essential oils of 3 *Eucalyptus* species. For the abbreviations of the Eucalyptus species (▲), the arboreta and the compounds (●), refer Tables 1 and 3 for more detail.

Figure 2 Dendrogram obtained by cluster analysis based on the Euclidean distances between groups of the leaf essential oils of 3 Tunisian *Eucalyptus* species. Components that characterize the major subgroups, considered as chemotypes, are indicated. JBA = Jbel abderrahman
Table 3 Percentage content of the 24 Compounds Selected for the Principal Component and the Hierarchical Cluster Analyses in the Essential Oils Extracted from the Leaves of 3 Eucalyptus species harvested from six arboreta

| Compound                          | Abbreviation | Souriaiat | Zeria | Pyjar Choucha (Jenane) | Milik | Korboos | Pyjar Choucha (Jenane) | Milik | Korboos | Souariat |
|-----------------------------------|--------------|-----------|-------|------------------------|-------|---------|------------------------|-------|---------|---------|
| α-Pinene                          | α-pin        | 5.1±0.8  | 5.2±0.6 | 5.7±1.4                | 21.8±4.7 | 30.0±0.0 | 14.0±1.7               | 8.3±0.4 | 2.9±1.4 | 2.5±0.0 | 2.1±0.0 | 5.1±4.0 |
| Limonene                          | lim          | 4.3±0.8  | 3.8±0.0 | 3.3±0.0                | 1.0±0.0 | 1.9±0.0 | 0.6±0.0 | 3.9±0.5 | 1.7±0.7 | 1.0±0.0 | 1.5±0.0 | 3.3±1.7 |
| 1.8-Cineole                       | 1.8-cin      | 69.8±2.19| 70.7±1.5 | 71.8±0.6               | 40.1±5.79 | 39.1±0.0 | 47.6±1.2      | 66.7±9.1  | 71.2±6.6 | 64.2±0.0 | 79.4±0.0 | 72.3±2.7 |
| γ-Terpinene                       | γ-terp       | 0.1±0.0  | 0.1±0.0  | 0.1±0.0               | 0.1±0.0 | 0.1±0.0 | 0.3±0.0 | 0.3±0.7 | 0.3±0.7 | 0.1±0.0 | 0.1±0.0  |
| p-Cymene                          | p-cym        | 0.2±0.0  | 0.2±0.0  | 0.2±0.0               | 0.2±0.0 | 0.2±0.0 | 0.2±0.0 | 0.2±0.0 | 0.2±0.0 | 0.2±0.0 | 0.2±0.0  |
| β-Carophyllene                    | β-car        | 0.6±0.0  | 0.6±0.0  | 0.6±0.0               | 0.6±0.0 | 0.6±0.0 | 0.6±0.0 | 0.6±0.0 | 0.6±0.0 | 0.6±0.0 | 0.6±0.0 |
| Terpinene-4-ol                    | ter-4-ol     | 0.1±0.0  | 0.1±0.0  | 0.1±0.0               | 0.1±0.0 | 0.1±0.0 | 0.1±0.0 | 0.1±0.0 | 0.1±0.0 | 0.1±0.0 | 0.1±0.0 |
| Aromadendrene                     | aro          | 0.1±0.0  | 0.1±0.0  | 0.1±0.0               | 0.1±0.0 | 0.1±0.0 | 0.1±0.0 | 0.1±0.0 | 0.1±0.0 | 0.1±0.0 | 0.1±0.0 |
| allo-Aromadendrene                | allo-allo    | 0.1±0.0  | 0.1±0.0  | 0.1±0.0               | 0.1±0.0 | 0.1±0.0 | 0.1±0.0 | 0.1±0.0 | 0.1±0.0 | 0.1±0.0 | 0.1±0.0 |
| pinocarvone                       | pin          | 0.1±0.0  | 0.1±0.0  | 0.1±0.0               | 0.1±0.0 | 0.1±0.0 | 0.1±0.0 | 0.1±0.0 | 0.1±0.0 | 0.1±0.0 | 0.1±0.0 |
| α-Terpsteny-3-acetate             | α-ter        | 2.6±0.6  | 2.1±0.6  | 2.0±2.6               | 2.0±2.6 | 2.0±2.6 | 2.0±2.6 | 2.0±2.6 | 2.0±2.6 | 2.0±2.6 | 2.0±2.6 |
| α-Terpineol                       | α-ter        | 8.8±1.1  | 7.5±0.6  | 6.4±0.7               | 6.4±0.7 | 6.4±0.7 | 6.4±0.7 | 6.4±0.7 | 6.4±0.7 | 6.4±0.7 | 6.4±0.7 |
| Bicyclogermacene                  | Bicyclogermacene | 0.2±0.2  | 0.2±0.2  | 0.2±0.2               | 0.2±0.2 | 0.2±0.2 | 0.2±0.2 | 0.2±0.2 | 0.2±0.2 | 0.2±0.2 | 0.2±0.2 |
| trans-p-Mentha-1(7).8dien-2-ol     | trans-p-Mentha-1(7).8dien-2-ol | 0.7±0.5  | 0.8±0.5  | 0.9±0.5               | 0.9±0.5 | 0.9±0.5 | 0.9±0.5 | 0.9±0.5 | 0.9±0.5 | 0.9±0.5 | 0.9±0.5 |
| cis-p-Mentha-1(7).8dien-2-ol       | cis-p-Mentha-1(7).8dien-2-ol | 0.7±0.5  | 0.8±0.5  | 0.9±0.5               | 0.9±0.5 | 0.9±0.5 | 0.9±0.5 | 0.9±0.5 | 0.9±0.5 | 0.9±0.5 | 0.9±0.5 |
| Epiglobolol                       | Epiglobolol  | 0.1±0.0  | 0.1±0.0  | 0.1±0.0               | 0.1±0.0 | 0.1±0.0 | 0.1±0.0 | 0.1±0.0 | 0.1±0.0 | 0.1±0.0 | 0.1±0.0 |
| Globolol                          | Globolol     | 0.5±0.0  | 0.5±0.0  | 0.5±0.0               | 0.5±0.0 | 0.5±0.0 | 0.5±0.0 | 0.5±0.0 | 0.5±0.0 | 0.5±0.0 | 0.5±0.0 |
| Viridiflorol                      | Viridiflorol | 0.2±0.0  | 0.2±0.0  | 0.2±0.0               | 0.2±0.0 | 0.2±0.0 | 0.2±0.0 | 0.2±0.0 | 0.2±0.0 | 0.2±0.0 | 0.2±0.0 |
| Guaiol                            | Guaiol       | 0.1±0.0  | 0.1±0.0  | 0.1±0.0               | 0.1±0.0 | 0.1±0.0 | 0.1±0.0 | 0.1±0.0 | 0.1±0.0 | 0.1±0.0 | 0.1±0.0 |
| Eremophilenol                     | Eremophilenol | 0.1±0.0  | 0.1±0.0  | 0.1±0.0               | 0.1±0.0 | 0.1±0.0 | 0.1±0.0 | 0.1±0.0 | 0.1±0.0 | 0.1±0.0 | 0.1±0.0 |
| Spathulenol                       | Spathulenol  | 0.2±0.0  | 0.2±0.0  | 0.2±0.0               | 0.2±0.0 | 0.2±0.0 | 0.2±0.0 | 0.2±0.0 | 0.2±0.0 | 0.2±0.0 | 0.2±0.0 |
| iso-Spathulenol                   | iso-Spathulenol | 0.2±0.0  | 0.2±0.0  | 0.2±0.0               | 0.2±0.0 | 0.2±0.0 | 0.2±0.0 | 0.2±0.0 | 0.2±0.0 | 0.2±0.0 | 0.2±0.0 |
| β-Eudesmol                       | β-Eudesmol | 0.1±0.0  | 0.1±0.0  | 0.1±0.0               | 0.1±0.0 | 0.1±0.0 | 0.1±0.0 | 0.1±0.0 | 0.1±0.0 | 0.1±0.0 | 0.1±0.0 |

Value followed by the different letter in same vertical column is significantly different according to Duncan’s multiple range test (P < 0.05)
3.3 Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA)

To evaluate the effect of the zone of the harvest (arboreta) on the essential oil chemical composition of the studied *Eucalyptus* species populations, 24 compounds with contents in the essential oils of minimum 0.5% in at least one species (Table 3), were selected for the PCA and the HCA. The contents of the selected oil components were significantly different between the species (p<0.05). The PCA horizontal axis explained 43.04% of the total variance and the vertical axis a further 15.76% (Figure 1). The HCA (Figure 2) based on the Euclidean distances between groups indicated three groups of species (A, B, and C), identified by their essential oil chemotypes with a dissimilarity >15 (Figure 2). Group C was further divided into two Subgroups (C1 and C2) within a dissimilarity >5.

*Group A* reduced to E. sideroxylon from Jbel Abderrahman arboreta, correlated positively with the two axes. It stands out forming a separate group in both HCA and PCA analysis. The essential oil of which was distinguished from the other provesences by the highest mean percentages in bicyclogermacrene (3.1±0.0%), isospithulenol (1.1±0.0%), globulol (4.7±0.0%), viridiflorol (1.8±0.0%), eremophile (0.7±0.0%), guaoil (0.6±0.0%), spathulenol (1.3±0.0%), allostaporene (0.9±0.0%). It was also characterized by the lowest content of α-pinene (2.5±0.0%) and by its relatively low percentage in trans-pinocarveol (1.8±0.0%). In comparison with the others provenance, The 1,8-cineole content of this oil was relatively medium (64.2±0.0%). It appears that the type of the soil such us sandy clay for hajeb laayoun arboreta had a significant influence on the biosynthesis of the chemical compound and could favourite ones and inhibits the synthesis of the others.

*Group B* is represented by oils of *E. astringens* from Korbous, Sejneane and Mrifek arboreta, correlating positively and negatively with both axes. They are characterized by the highest content in α-pinene (30.0±0.0, 14.0±1.7, 21.8±4.7% respectively), pinocarvone (0.8±0.0, 3.7±0.6, 2.9±0.84; respectively) trans-pinocarveol (3.7±0.0, 9.3±1.2, 10.0±2.5% respectively), aromadendrene (3.8±0.0%) for those from Korbous and Sejneane arboreta and 4.9±2.1% for the provenance Mrifek arboreta, epiglobulol (0.9±0.0 - 1.3±0.6%), globulol (4.5±0.0, 5.3±0.6, 5.7±1.3%; respectively), viridiflorol (0.9-1.0 %) They are also characterized by the lowest mean percentages in 1,8-cineole (39.1±0.0, 47.6±1.2, 40.1±5.79 %; respectively). The variation between these three sources was mainly due to the variation in the content of these compounds, such as α-pinene which distinguish essentially the provenance of Korbous and Sejneane, while the content of tr-pinocarveol, pinocarvone and 1,8-cineole was more abundant in oils obtained from Mrifek and Sejneane sources. *Sub-group C1*, formed by E. sideroxylon from Korbous and Sejneane, their essential oils are characterized by the highest content in 1,8-cineole (79.4±0.0, 71.2±3.6%; respectively) and by lowest mean percentage in α-pinene (2.5±0.0, 2.9±1.4%; respectively). However they are also characterized by a relatively high levels of p-cymene, tr-mentha-1 (7) , 8dien -2 -ol, cis -p- mentha -1 (7) , 8dien -2 -ol and β-eudesmol.

*Subgroup C2* represented by oils of *E. sideroxylon* of Souiniat and Mrifek arboreta and by those of *E. cineera* from Souiniat, Sejane and Zerniza arboreta. The essential oils of which were characterized by mean percentages of 1,8-cineole relatively comparable to those of the sub-group C1 (66.7±9.1 to 72.3±2.7% ). They were characterized also by the highest content in α-pinene (5.1±0.8-8.3±6.4%), limonene (3.3±0.0-4.3±0.8%) and α-terpinyl acetate (2.0±2.0-2.9±0.8%), while *E. cineera* representing the second species of this subgroup was distinguished from the other by its highest mean percentage in α -terpinene (6.4±0.7 to 8.8±1.1%).

Results of this study are in agreement with the findings of previous researches (Li et al., 1994; Li & Madden, 1995; Bignell et al., 1997; Della Porta et al., 1999; Tsi et al., 2003; Marzouki et al., 2009), the chemical composition of the essential oils varied significantly within species and environmental conditions. Zrira et al. (1994) found that the mean percentage of 1,8-cineole of *E. sideroxylon* essential oil varied from 76.9% for those from Jbilet region to 80.9% for those obtained from Tekerkoust area. This variation was also proved within the essential oils of *E. astringens*, mainly in the mean percentages of the 1,8-cineole (59.3-61.4%) and α-pinene (4.9-14.3%) for the provenances of Jbilet and Tekerkoust; respectively. Grbović et al. (2010) confirmed also this variation within some components of the essential oil of *E. camaldulensis*, such as p-cymene (17.38 – 28.60%) for the regions of Herceg Novi and Tivat; respectively, β-pinene (0.94, 11.48%) for Tivat and Sutomore; respectively, spathulenol (7.83, 14.15%) for Tivat and Herceg Novi; respectively and cryptone (4.97, 7.25%) for Kotor and Tivat regions; respectively.

Dunlop et al. (2003) have also demonstrated this variability within *E. Bicostata*, cultivated in 12 regions in Australia. They found that 1.8-cineole varied significantly from 44.0% for leaves collected from Mount area Bryan (B1) to 71.7% for those from Wittunga (B8) arboreta. It was the same for α-pinene (1.5 – 19.6%) for samples collected from wittinga (B9) and Flinders Island (B3) arboreta, respectively and for aromadendrene (5.4-15.2%) for those obtained from Wittunga (B8) and Mount Bryan (B1) arboreta respectively.

Conclusions

The essential oils obtained from three *Eucalyptus* species acclimated in six arboreta were characterized. They varied significantly within species and within their origin of harvest. Four chemotypes were defined, with a great variability between them. *E. cineera* grown in Pryor Choucha (Sejneane) arboreta was characterized by the highest mean oil yield,
whereas *E. sideroxylo* from Korbous and Sejnane arboreta contained the highest mean percentage in 1,8-cineole.

**Conflict of interest**

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

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