# CHEMODIVERSITY OF HELICHRYSUM ITALICUM (ROTH) G. DON SUBSP. ITALICUM ESSENTIAL OILS FROM BOSNIA AND HERZEGOVINA

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# CHEMODIVERSITY OF *HELICHRYSUM ITALICUM* (ROTH) G. DON SUBSP. *ITALICUM* ESSENTIAL OILS FROM BOSNIA AND HERZEGOVINA

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## ABSTRACT

The chemical composition of essential oil samples isolated from immortelle (*Helichrysum italicum* (Roth.) G. Don. subsp. *italicum)* collected on ten locations in Bosnia and Herzegovina was determined by GC/MS analysis. The whole wild population growing on different altitudes and soil types is covered in flowering period, with the aim of determining chemotype of EOs. Each of the examined EOs was characterized by a high content of sesquiterpene hydrocarbons (mean value 32.5 %), followed by oxigenated monoterpenes (20.9 %), monoterpene hydrocarbons (16.3 %), diketones (17.9 %), non-terpene esters (5.5 %) and oxigenated sesquiterpenes (5.0 %). The main compounds were *γ*-curcumene (12.6 %), neryl acetate (11.5 %), *α*-pinene (10.7 %) and 4,6,9-trimethyldec-8-en-3,5-dione (7.9%). To compare the chemical variability of the immortelle EOs from B&H with the other Mediterranean areas, studied previously, multivariate statistical analysis was performed (PCA and CA). The essential oils from B&H in chemical composition are similar to those from the Adriatic region (Croatia and southeast Italy), and different from Tyrrhenian islands' oils. These EOs were distinguished by the content of *γ*- and *β*-curcumene, *α*-bergamotene and italicene. The essential oils isolated from B&H immortelle possess specific chemical composition which gives them high quality and geographic distinctiveness.

**KEYWORDS**:
*Helichrysum italicum,* essential oil, chemodiversity, terpenoids, *γ*-curcumene

## INTRODUCTION

Immortelle (*Helichrysum italicum* (Roth.) G. Don. subsp. *italicum*)) is a medicinal aromatic plant from the Asteraceae family. It grows wild in the regions of North Africa and the countries of southern Europe; Greece, France, Italy, Spain, Croatia and Bosnia and Herzegovina. In recent years, there is a large interest in the pharmaceutical, cosmetic and perfume industry for essential oil immortelle. Essential oil and extracts of immortelle have antioxidant, antibacterial, anti-inflammatory, fungicidal and other beneficial properties. [1] The use of essential oil of immortelle depends in the first place on its chemotype and the main components of the essential oil. For example, nerol and its derivatives are preferred in the perfume industry, while curcumene oil chemotype is more widely used in the food industry. [2] The high content of *β*-diketones is appropriate for the pharmaceutical industry. The content of components in the essential oil depends primarily on the genotype and environmental conditions in which the plant grows [3]. Lack of knowledge about the chemical composition and pharmacological properties of plants from Bosnia and Herzegovina (B&H) is one of the major threats to their sustainable use.

The chemical study of the immortelle (*Helichrysum italicum* (Roth. G. Don. subsp. *italicum*)) essential oils from different collecting sites is the main key to the insight of variations and diversities of its chemical compounds and to determination of chemotypes. Previous immortelle essential oil studies from the area of B&H pointed to the differences in the chemical composition depending on the growing stage and part of plants [4]. The aim of this study was to conduct an analysis of wild growing immortelle essential oil from different areas of B&H, and to compare their composition with essential oils from other Mediterranean regions. The fact of knowing the plant chemotype and the environmental factors of cultivation can facilitate the seeds selection or cuttings for plantation cultivation of the plants with the targeted chemical profile.

## MATERIALS AND METHODS

**Isolation of the Essential Oil.** The wild growing immortelle was collected during the flowering period on ten locations in B&H (June, 2016). Species were identified by professor of Botany PhD Anđelka

Lasić and stored at the Department of Biology, Faculty of Science and Education, University of Mostar. The localities studied in this research, their coordinates, altitude and soil type are given in Table 1. The plant material was air-dried at ambient temperature without exposure to direct sunlight. Dry aerial parts of the immortelle (100 g) and distilled water (500 mL) were placed in a Clevenger type apparatus. The hydro distillation was conducted for two hours. The obtained essential oil separated and dried with anhydrous sodium sulphate. The yield was determined by the gravimetric method. The essential oil was stored in a sealed vial at 4°C until the analysis. Isolation of the essential oil from each sample and analysis were carried out in triplicate.

**Gas Chromatography–Mass Spectrometry.** Analysis of the essential oils were carried out using Shimadzu GC/MS QP2010 system equipped with an AOC–20i autosampler, using two fused silica capillary columns with different polarity. The non-polar column was Inert Cap (5% diphenyl – 95% dimethylpolysiloxane, 30 m × 0.25 mm i.d., film thickness 0.25 mm) and the polar column was Rtx–Wax (polyethylene glycol, 30 m × 0.25 mm × 0.25 mm). The operating conditions for non-polar column were as follows: injection volume: 1.0 mL of solution diluted 1:500 v/v in pentane; injection mode: splitless; injection temperature: 250 °C; carrier gas: helium, 1.15 mL/min; oven temperature program: 70 °C (1.5 min), 70 – 120 °C (5 °C/min), 120 – 240 °C (4 °C min), 240 °C (2 min). For polar column operating conditions were as follows: flow rate of carrier gas: 1.21 mL/min; oven temperature program: 60 °C (2 min), 60 – 240 °C (3 °C/min), 240 °C (10 min). MS conditions: ion source temperature: 250 °C, ionization voltage: 70 eV, mass range: m/z 40 – 400 u. GCMSolution 2.5 (Shimadzu) was used to handle data. All analyses were done in triplicate.

Identification of oil components was based on retention indices on a polar and non-polar column relative to a homologous series of *n*-alkanes (C8 – C40), literature data [5,6,7,8] and on comparison of their mass spectra with the NIST and Wiley spectra library.

**Statistical methods.** Chemical composition of 10 immortelle essential oils from B&H were compared with 64 oils from Algeria, B&H, Croatia, France, Greece, Italy and Montenegro (Table 3). Essential oils selected for comparison were similar in extraction and determination mode, in the fact that all of the plant material was collected during the flowering stage, furthermore, oils were extracted by hydro-distillation and analyzed by GC with MS or FID detector. The principal component analysis (PCA) was used to reveal the relations between regions of immortelle habitat. 74 oils were actively observed and 22 compounds most frequently found in the immortelle oil, which were selected as active variables. For the missing data the median of the substance was used. PCA was performed by singular value decomposition which examines the covariances/correlations between individuals using factoextra package [9] of the R statistical program [10]. The hierarchical cluster analysis on principal components (HCPC) was used to group the oils. The principal components, obtained by principal component analysis, were analyzed by hierarchical clustering in order to reduce the number of variables and to reduce the noise. The HCPC analysis was performed by FactoMineR package [11] of the R program.

## RESULTS AND DISCUSSION

**The immortelle essential oils from Bosnia and Herzegovina.** Immortelle (*Helichrysum italicum* (Roth. G. Don. Subsp. *italicum*)) was collected during the period of flowering on ten sites in B&H, where it grows wild in poor limestone soils. The wild populations of these plants are not represented on the territory of Bosnia and all localities in Herzegovina were studied. The research covered all areas where immortelle appears from sea level (7 m) to the highlands (761 m). The soil types in the studied sites are terra rosa, calcocambisol, calcomelanosol, or any combination thereof. Young aboveground parts of the plant (leaves, stems and flowers) were subjected to distillation. The yields of essential oils were in the range of 0.15 – 0.32% (table 1). Relatively low essential oil yields of this Mediterranean species have been reported in previous similar studies [12,13,14] .

The chemical composition of immortelle essential oils from different locations in B&H and descriptive statistics are given in Table 2. In total, 61 components identified in immortelle essential oil, representing 97.0 – 98.8 % of the whole oil composition. Two columns of different polarity were used. Percentages and order of evaluation are given in the apolar column. The essentials oils showed similar values of arithmetic mean and median for all compounds quantified indicating high homogeneity of the oils. The main components in all of the analyzed oils are monoterpene, sesquiterpene and $\beta$-diketones. Their mean values are the following: for sesquiterpene hydrocarbons (32.5 %), oxigenated monoterpenes (20.9 %), monoterpene hydrocarbons (16.3 %), diketones (17.9 %), non-terpene esters (5.5 %) and for oxigenated sesquiterpenes (5.0 %). Largest content of monoterpene hydrocarbons were $\alpha$-pinene (4.1 - 19.3%) and limonene (1.3 – 6.2%). Neryl acetate (3.6-21.1%) and linalool (2.3-5.7%) were the most oxygenated monoterpenes. The most represented sesquiterpene hydrocarbons were $\gamma$-curcumene (8.4-18.6%), *trans*-caryophyllene (3.1-8.5%) and $\beta$-selinene (2.8-6.7%). 4,6,9-Trimethyldec-8-en-3,5-dione (5.1-14.5%) was the most represented

**TABLE 1**
**Geographical origin of the analyzed immortelle essential oils from B&H**

| Location number | Location | GPS Coordinates | Altitude | Soil | Voucher specimens | Essential oil yields[a] |
|---|---|---|---|---|---|---|
| 1. | Mostar | N 43°18'12.73'' E 17°49'22.54'' | 55 m | Terra rossa | FPMOZ-SB-11-2016 | 0.21 % |
| 2. | Blatnica | N 43°15'51.92'' E 17°42'2.83'' | 377 m | Terra rossa | FPMOZ-SB-12-2016 | 0.17 % |
| 3. | Crnač | N 43°24'53.89'' E 17°35'46.81'' | 449 m | Calcocambisol | FPMOZ-SB-13-2016 | 0.29 % |
| 4. | Britvica | N 43°25'35.64'' E 17°32'4.33'' | 761 m | Calcomelanosol | FPMOZ-SB-14-2016 | 0.28 % |
| 5 | Neum | N 42°55'0'' E 17°37'0'' | 7 m | Calcocambisol + Terra Rossa | FPMOZ-SB-15-2016 | 0.21 % |
| 6. | Hutovo | N 42°57'29.56'' E 17°48'10.44'' | 393 m | Calcomelanosol + Calcocambisol | FPMOZ-SB-16-2016 | 0.30 % |
| 7. | Crnići | N 43°7'32.2'' E 17°51'14.57'' | 265 m | Calcocambisol + Terra Rossa | FPMOZ-SB-17-2016 | 0.32 % |
| 8. | Ljubinje | N 42°57'17.39'' E 18°5'10.0 7'' | 487 m | Terra rossa | FPMOZ-SB-18-2016 | 0.15 % |
| 9. | Žakovo | N 42°48'40.59'' E 18°7'14.23'' | 256 m | Terra rossa + Calcocambisol | FPMOZ-SB-19-2016 | 0.26 % |
| 10. | Trebinje | N 42°42'17.59'' E 18°19'37.72'' | 275 | Calcomelanosol | FPMOZ-SB-20-2016 | 0.19 % |

[a] The yields are averages of three replicates of distillation plant material

**TABLE 2**
**Chemical composition of immortelle (*Helichrysum italicum* (Roth.) G. Don. subsp. *italicum*) essential oils from different locations in Bosnia and Herzegovina and descriptive statistics**

| | | Locations | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Compound | RI[a] | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | mean | median | min | max |
| *α*-Pinene | 935 | 7.0 | 9.2 | 19.2 | 13.2 | 19.3 | 4.1 | 7.8 | 17.5 | 4.1 | 5.2 | 10.7 | 8.5 | 4.1 | 19.3 |
| *β*- Pinene | 981 | 0.0 | 0.1 | 0.3 | 0.2 | 0.4 | 0.1 | 0.1 | 0.0 | 0.2 | 0.0 | 0.1 | 0.1 | 0.0 | 0.4 |
| *β*-Myrcene | 987 | 0.6 | 0.3 | 0.2 | 1.1 | 0.6 | 0.4 | 0.4 | 0.1 | 0.3 | 0.1 | 0.4 | 0.3 | 0.1 | 1.1 |
| *α*-Terpinene | 1022 | 0.0 | 0.0 | 0.2 | 0.2 | 0.3 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.1 | 0.1 | 0.0 | 0.3 |
| Limonene | 1030 | 3.1 | 2.8 | 4.8 | 6.2 | 5.2 | 4.6 | 4.4 | 1.3 | 3.1 | 2.8 | 3.8 | 3.8 | 1.3 | 6.2 |
| *γ*-Terpinene | 1056 | 0.4 | 0.6 | 1.7 | 1.6 | 1.2 | 0.7 | 0.4 | 0.4 | 0.4 | 0.4 | 0.8 | 0.5 | 0.3 | 1.7 |
| α-Terpinolene | 1088 | 0.2 | 0.3 | 0.3 | 0.3 | 0.8 | 0.1 | 0.4 | 0.3 | 0.5 | 0.3 | 0.3 | 0.3 | 0.1 | 0.8 |
| **TMH** | | **11.3** | **13.3** | **26.8** | **22.8** | **27.8** | **10.0** | **13.5** | **19.7** | **8.5** | **8.9** | **16.3** | **13.4** | **8.5** | **27.8** |
| Linalool | 1099 | 3.9 | 4.4 | 4.2 | 4.7 | 4.8 | 2.8 | 2.3 | 2.9 | 5.7 | 2.5 | 3.8 | 4.0 | 2.3 | 5.7 |
| D-Fenchyl alcohol | 1120 | 0.1 | 0.3 | 0.1 | 0.1 | 0.2 | 0.1 | 0.1 | 0.2 | 0.2 | 0.2 | 0.2 | 0.1 | 0.1 | 0.2 |
| *trans*-Pinocarveol | 1143 | 0.2 | 0.2 | 0.1 | 0.1 | 0.1 | 0.2 | 0.1 | 0.4 | 0.2 | 0.1 | 0.2 | 0.1 | 0.1 | 0.4 |
| Borneol | 1172 | 0.2 | 0.5 | 0.2 | 0.3 | 0.3 | 0.2 | 0.2 | 0.6 | 0.4 | 0.4 | 0.3 | 0.3 | 0.2 | 0.6 |
| *α*-Terpineol | 1195 | 2.1 | 2.2 | 1.4 | 2.3 | 2.0 | 1.5 | 1.7 | 1.9 | 2.3 | 1.7 | 1.9 | 1.9 | 1.4 | 2.3 |
| Nerol | 1222 | 1.0 | 1.4 | 2.0 | 3.1 | 2.2 | 0.8 | 1.5 | 0.1 | 1.0 | 3.4 | 1.6 | 1.4 | 0.1 | 3.4 |
| Thymol | 1295 | 0.0 | 0.0 | 0.0 | 0.6 | 0.3 | 0.0 | 0.0 | 0.0 | 0.2 | 0.0 | 0.1 | 0.0 | 0.0 | 0.6 |
| Neryl acetate | 1356 | 13.8 | 12.0 | 11.2 | 21.1 | 13.7 | 12.2 | 13.0 | 3.6 | 6.8 | 7.8 | 11.5 | 12.1 | 3.6 | 21.1 |
| Neryl propionate | 1446 | 1.6 | 1.4 | 1.2 | 1.7 | 1.2 | 0.4 | 1.6 | 0.1 | 0.4 | 2.4 | 1.2 | 1.3 | 0.1 | 2.4 |
| **TOM** | | **22.9** | **22.3** | **20.4** | **33.9** | **24.8** | **18.3** | **20.6** | **9.8** | **17.1** | **18.5** | **20.9** | **20.5** | **9.8** | **33.9** |
| *α*-Ylangene | 1368 | 0.4 | 0.4 | 0.2 | 0.2 | 0.2 | 0.4 | 0.3 | 0.7 | 0.4 | 0.5 | 0.4 | 0.4 | 0.2 | 0.7 |
| *α*-Copaene | 1375 | 2.9 | 3.0 | 1.0 | 1.2 | 1.2 | 2.0 | 2.0 | 4.0 | 2.6 | 3.3 | 2.3 | 2.3 | 1.0 | 4.0 |
| Italicene | 1405 | 2.3 | 3.4 | 1.4 | 1.7 | 1.4 | 2.6 | 3.4 | 2.1 | 2.3 | 2.9 | 2.3 | 2.3 | 1.4 | 3.4 |
| *α*-Bergamotene | 1412 | 0.9 | 0.9 | 0.6 | 0.8 | 0.7 | 0.3 | 0.7 | 0.9 | 0.6 | 0.7 | 0.7 | 0.7 | 0.3 | 0.9 |
| *trans*-Caryophyllene | 1420 | 5.4 | 4.3 | 3.1 | 4.0 | 4.1 | 4.4 | 4.3 | 8.5 | 4.5 | 5.3 | 4.8 | 4.4 | 3.1 | 8.5 |
| *β*- Farnesene | 1451 | 0.1 | 0.1 | 0.2 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.2 | 0.1 | 0.1 | 0.1 | 0.2 |

| Compound | RI[a] | Locations 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | mean | median | min | max |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *α*-Humulene | 1455 | 0.2 | 0.2 | 0.2 | 0.1 | 0.1 | 0.2 | 0.2 | 0.3 | 0.2 | 0.2 | 0.2 | 0.2 | 0.1 | 0.3 |
| Aromadendrene | 1459 | 0.1 | 0.1 | 0.0 | 0.0 | 0.0 | 0.1 | 0.1 | 0.2 | 0.1 | 0.2 | 0.1 | 0.1 | 0.0 | 0.2 |
| *β*-Acoradiene | 1466 | 0.1 | 0.2 | 0.0 | 0.1 | 0.0 | 0.2 | 0.2 | 0.1 | 0.1 | 0.2 | 0.1 | 0.1 | 0.0 | 0.2 |
| *γ* -Curcumene | 1477 | 11.9 | 12.2 | 8.4 | 9.7 | 9.4 | 16.2 | 12.3 | 10.0 | 1.2 | 18.6 | 12.6 | 12.1 | 8.4 | 18.6 |
| *ar*-Curcumene | 1483 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.1 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.1 | 0.2 |
| *β*-Selinene | 1489 | 5.6 | 4.2 | 5.6 | 2.8 | 3.4 | 2.8 | 4.7 | 5.3 | 6.7 | 3.6 | 4.5 | 4.5 | 2.8 | 6.7 |
| *α*-Selinene | 1495 | 3.5 | 2.6 | 3.4 | 1.8 | 2.1 | 1.8 | 3.0 | 4.4 | 4.1 | 2.3 | 2.9 | 2.8 | 1.8 | 4.4 |
| *β*-Bisabolene | 1508 | 0.2 | 0.3 | 0.2 | 0.3 | 0.2 | 0.3 | 0.3 | 0.3 | 0.3 | 0.5 | 0.3 | 0.3 | 0.2 | 0.4 |
| ϒ-Cadinene | 1512 | 0.5 | 0.5 | 0.3 | 0.2 | 0.2 | 0.6 | 0.5 | 0.4 | 0.6 | 0.6 | 0.4 | 0.5 | 0.2 | 0.6 |
| δ-Cadinene | 1517 | 0.7 | 0.5 | 0.2 | 0.1 | 0.2 | 0.3 | 0.3 | 0.8 | 0.5 | 0.7 | 0.4 | 0.4 | 0.1 | 0.8 |
| **TSH** | | **35.0** | **33.4** | **25.1** | **23.3** | **2.5** | **32.3** | **32.7** | **38.5** | **40.6** | **40.2** | **32.5** | **33.1** | **23.3** | **40.6** |
| Italicene ether | 1533 | 0.1 | 0.3 | 0.2 | 0.1 | 0.4 | 0.6 | 0.4 | 0.4 | 0.2 | 0.3 | 0.3 | 0.3 | 0.1 | 0.6 |
| d-Nerolidol | 1559 | 0.2 | 0.2 | 1.5 | 0.0 | 0.0 | 0.1 | 0.1 | 0.0 | 0.1 | 0.2 | 0.2 | 0.1 | 0.0 | 1.5 |
| *α*-Caryophyllene alcohol | 1573 | 0.0 | 0.2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.2 |
| Cariophyllene oxide | 1580 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.1 | 0.1 | 0.1 | 0.0 | 0.1 | 0.1 | 0.1 | 0.0 | 0.1 |
| Guaiol | 1594 | 0.2 | 0.1 | 0.2 | 0.0 | 0.2 | 0.2 | 0.5 | 0.4 | 0.0 | 0.2 | 0.2 | 0.2 | 0.0 | 0.5 |
| Geranyl isovalerate | 1603 | 0.2 | 0.3 | 0.1 | 0.0 | 0.1 | 0.2 | 0.1 | 0.1 | 0.3 | 0.4 | 0.2 | 0.1 | 0.0 | 0.4 |
| Viridiflorol | 1607 | 1.7 | 1.5 | 0.2 | 0.3 | 0.4 | 0.9 | 1.1 | 2.5 | 1.0 | 1.9 | 1.2 | 1.1 | 0.2 | 2.5 |
| Rosifoliol | 1611 | 0.5 | 0.5 | 0.7 | 0.2 | 0.6 | 0.9 | 2.0 | 0.6 | 0.2 | 0.6 | 0.6 | 0.6 | 0.1 | 2.0 |
| Cubenol | 1626 | 0.1 | 0.2 | 0.0 | 0.0 | 0.0 | 0.1 | 0.1 | 0.3 | 0.0 | 0.1 | 0.1 | 0.1 | 0.0 | 0.3 |
| *γ*-Eudesmol | 1630 | 0.0 | 0.1 | 0.1 | 0.0 | 0.0 | 0.0 | 0.1 | 0.1 | 0.0 | 0.1 | 0.1 | 0.0 | 0.0 | 0.1 |
| *β*-Eudesmol | 1634 | 0.2 | 0.1 | 0.3 | 0.1 | 0.2 | 0.6 | 0.5 | 0.4 | 0.1 | 0.3 | 0.3 | 0.3 | 0.1 | 0.6 |
| *τ*-Cadinol | 1639 | 0.2 | 0.2 | 0.0 | 0.0 | 0.0 | 0.1 | 0.1 | 0.3 | 0.1 | 0.2 | 0.1 | 0.1 | 0.0 | 0.3 |
| *α*-Eudesmol | 1653 | 0.7 | 0.4 | 0.5 | 0.2 | 0.3 | 0.8 | 0.9 | 1.1 | 0.3 | 0.6 | 0.6 | 0.6 | 0.2 | 1.1 |
| Juniper camphor | 1656 | 0.5 | 0.7 | 0.7 | 0.3 | 0.5 | 0.5 | 0.9 | 1.0 | 0.9 | 0.6 | 0.6 | 0.6 | 0.3 | 0.9 |
| *β*- Bisabolol | 1668 | 0.1 | 0.2 | 0.1 | 0.1 | 0.2 | 0.4 | 0.4 | 0.3 | 0.2 | 0.4 | 0.2 | 0.2 | 0.1 | 0.4 |
| *α*-Bisabolol | 1683 | 0.1 | 0.2 | 0.1 | 0.1 | 0.1 | 0.2 | 0.2 | 0.2 | 0.1 | 0.2 | 0.1 | 0.1 | 0.1 | 0.2 |
| **TOS** | | **4.8** | **5.3** | **4.6** | **1.5** | **3.2** | **5.7** | **7.6** | **7.7** | **3.4** | **6.2** | **5.0** | **5.1** | **1.5** | **7.7** |
| Isobuthyl 2-methyl-2-butanoate | 1045 | 0.6 | 0.5 | 0.6 | 0.9 | 0.4 | 0.7 | 0.7 | 1.3 | 0.4 | 0.4 | 0.6 | 0.6 | 0.4 | 1.3 |
| Pentyl 3-methyl-2-butanoate | 1149 | 2.6 | 2.8 | 5.0 | 2.3 | 3.4 | 2.8 | 2.6 | 5.9 | 2.2 | 1.5 | 3.1 | 2.7 | 1.5 | 5.8 |
| Hexyl 2-methylbutanoate | 1247 | 0.1 | 0.2 | 0.2 | 0.2 | 0.4 | 0.2 | 0.2 | 0.1 | 0.2 | 0.3 | 0.2 | 0.2 | 0.1 | 0.4 |
| Hexyl senecioate | 1281 | 0.9 | 1.0 | 1.4 | 1.5 | 2.4 | 2.2 | 2.2 | 1.6 | 0.9 | 1.5 | 1.6 | 1.5 | 0.9 | 2.4 |
| **TNE** | | **4.2** | **4.6** | **7.2** | **4.9** | **6.6** | **5.9** | **5.6** | **8.9** | **3.7** | **3.6** | **5.5** | **5.3** | **3.6** | **8.9** |
| 4,6-Dimethylocta -3,5-dione | 1180 | 4.7 | 4.2 | 3.5 | 4.0 | 3.2 | 2.0 | 2.3 | 1.8 | 3.3 | 4.1 | 3.3 | 3.4 | 1.8 | 4.7 |
| 4,6,9-Trimethyldec-8-en-3,5-dione | 1431 | 7.5 | 7.5 | 5.4 | 5.1 | 5.7 | 14.5 | 8.5 | 5.6 | 13.1 | 6.6 | 7.9 | 7.1 | 5.1 | 14.5 |
| 2,4,6,9-Tetramethyldec-8-en3,5-dione A* | 1473 | 5.2 | 4.5 | 3.0 | 1.8 | 2.8 | 6.1 | 4.0 | 3.2 | 6.0 | 5.5 | 4.2 | 4.3 | 1.8 | 6.1 |

| Compound | RI[a] | Locations 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | mean | median | min | max |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2,4,6,9-Tetramethyldec-8-en3,5-dione B* | 1480 | 1.5 | 1.2 | 0.6 | 0.7 | 0.8 | 0.7 | 2.2 | 1.3 | 1.1 | 0.9 | 1.1 | 0.9 | 0.6 | 2.2 |
| 5,7,10,-Trimethylundec-9-en 4,6-dione | 1502 | 0.3 | 0.4 | 0.3 | 0.2 | 0.2 | 0.7 | 0.4 | 0.2 | 0.5 | 0.4 | 0.4 | 0.4 | 0.2 | 0.7 |
| 3,5,7,10-Tetramethylundec-9-en-4,6-dione A* | 1564 | 0.7 | 0.4 | 0.5 | 0.2 | 0.1 | 0.5 | 0,6 | 0.1 | 0.5 | 0.9 | 0.5 | 0.5 | 0.1 | 0.9 |
| 3,5,7,10-Tetramethylundec-9-en-4,6-dione B* | 1569 | 0.7 | 0.5 | 0.5 | 0.2 | 0.2 | 0.5 | 0.6 | 0.1 | 0.5 | 1.0 | 0.5 | 0.5 | 0.1 | 1.0 |
| **TD** | | **20.6** | **18.8** | **13.9** | **12.1** | **13.0** | **25.0** | **18.6** | **12.2** | **24.9** | **19.4** | **17.9** | **18.7** | **12.1** | **25.0** |
| 2-Undecanone | 1290 | 0.0 | 0.1 | 0.1 | 0.0 | 0.1 | 0.0 | 0.1 | 0.3 | 0.1 | 0.2 | 0.1 | 0.1 | 0.0 | 0.3 |
| **TNTK** | | **0.0** | **0.1** | **0.1** | **0.0** | **0.1** | **0.0** | **0.1** | **0.3** | **0.1** | **0.2** | 0.1 | 0.1 | 0.0 | 0.3 |
| **TOTAL** | | **98.8** | **97.8** | **98.1** | **98.4** | **98.8** | **97.2** | **98.6** | **97.1** | **98.4** | **97.0** | **98.0** | **98.2** | **97.0** | **98.8** |

**Notes:** RI[a]= Retention index on apolar column; A* and B* are diastereomers [5,17]; **TMH =** Total monoterpenic hydrocarbons; **TOM =** Total oxygenated monoterpenes; **TSH** = Total sesquiterpenic hydrocarbons; **TOS** = Total oxygenated sesquiterpenes; **TNE** = Total non-terpenic esters; **TD** = Total diketones, **TNTK** = Total non-terpenic ketones.

**TABLE 3**
**Data of the immortelle essential oils used for comparison study**

| R | SY | C | NO | C /L | Oil extraction | I | Analytical column | CG |
|---|---|---|---|---|---|---|---|---|
| [14] | 2011 | Wild | 4 | Croatia /Brač, Biokovo, Tijarica, Makarska | Hydrodistillation | GC-MS | Fused silica capillary column HP-5 | He |
| [23] | 2015 | Plantation | 1 | Bosnia and Herzegovina/ Ljubuški, | Hydrodistillation Clevenger | GC- MS | HP-5MS (5%-phenyl-methylpolysiloxane), 30 m × 0.25 mm | He |
| [22] | 2013 | Wild | 1 | Algeria/ Bejaia, | Hydrodistillation Clevenger | GC- MS | Fused silica capillary column HP-5, 30 m × 0.25 mm | He |
| [2] | 2011 | Wild | 1 | Montenegro/ Valdanos | Hydrodistillation | GC- MS GC-FID | HP-5 MS capillary column, 30 m × 0.25 mm | He |
| [20] | 2013 | Wild | 7 | Italy /Elba | Hydrodistillation Clevenger | GC- MS GC-FID | Fused silica capillary columns HP-Wax and HP-5, 30 m × 0.25 mm | He $N_2$ |
| [19] | 2003 | Wild | 11 | Italy / Tuscan archipelago | Hydrodistillation Clevenger | GC- MS GC-FID | Fused silica capillary columns Rtx-1 (polydimethylsiloxane) and Rtx-Wax (polyethyleneglycol), 60 m × 0.22 mm | He |
| [21] | 1994 | Wild | 1 | Greece / Amorgos | Steam distillation | GC-FID | Fused silica capillary columns Supelcowax 10, 30 m × 0.32 i.d. and DB-5, 60 m × 0.25 i.d. | n.s. |
| [3] | n.s. | Plantation | 20 | Italy/five south regions France/Corsica | Steam distillation | GC-MS GC-FID | Fused silica capillary column DB-5, 30 m × 0.25 mm | n.s. H |
| [17] | 1999-2001 | Plantation | 7 | France /Corsica | Semi-industrial steam distillation, Hydrodistillation Clevenger | GC-MS GC-FID | Fused-silica capillary columns BP-1 (dimethylsiloxane) and BP-20 (polyethylene glycol), 50 m × 0.22 mm | He |
| [15] | 2007 | Wild | 1 | Croatia/Split | Hydrodistillation Clevenger | GC-MS GC-FID | Apolar column HP-101 (methyl silicone fluid), 25 m × 0.2 mm) and polar column HP-20 M (Carbowax), 50 m × 0.2 mm | He |
| [13] | 2002 | Wild | 1 | Croatia/Split | Hydrodistillation Clevenger | GC-MS | Apolar column HP-101 (methyl silicone fluid), 25 m × 0.2 mm and polar column HP-20 M (Carbowax), 50 m × 0.2 mm | He |
| [12] | n.s. | Wild | 9 | Croatia and Bosnia and Herzegovina | Hydrodistillation | GC-FID GC-MS | Fused silica capillary column CP-Sil 8CB 25 m × 0.32 mm | He |

**R =** Reference; **SY =** Sampling year; **C** = Cultivation; n.s. **NO =** Number of oils; **C/L** = Country /Locality, **I** = Instrument; **CG** = Carrier gas; – not specified.

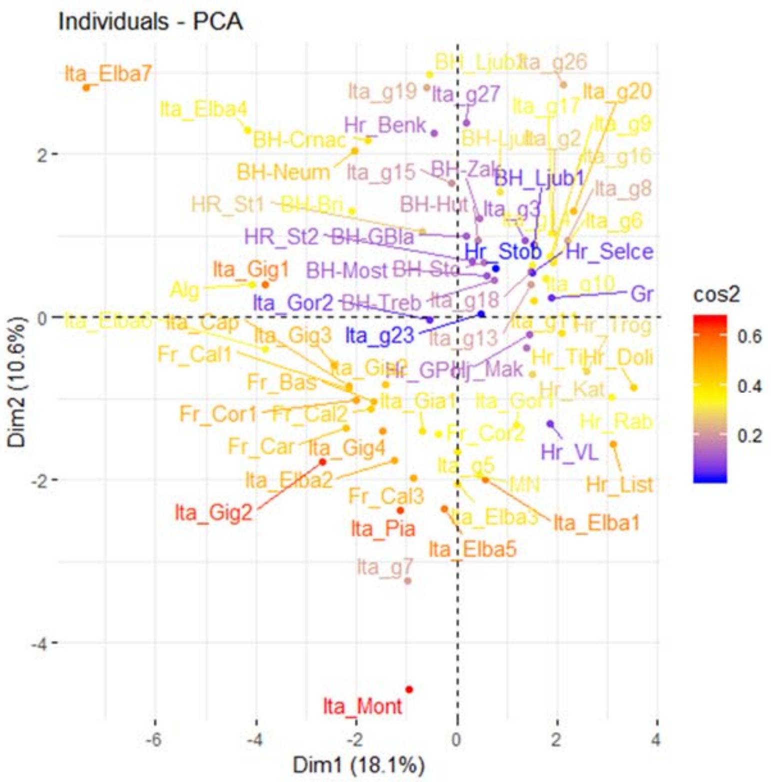


**FIGURE 1**

**Principal component analysis of 74 immortelle essential oils. The importance of principal components for the given oil as square cosine and represented by the color gradient of the label.**

Italian collection (Ita_g2-g27); Corsica, France (Fr_Cal1, Cal2, Car, Cal3, Cor1, Bas, Cor2); Croatia (Hr_Vl, Sto, Ben, GPolj, Doli, List, Tro, Rab, Sel, Kat, Tij, Mak, Sp1, Sp2); Greece (Gr); Algeria (Alg); Montenegro (MN); Elba Italy (Ita_Elba1-Elba7); Tuscan archipelago, Italy (Ita_Gor1 Gor2, Gia1, Gia2, Gig1, Gig2, Gig3, Gig4, Cap, Mont, Pia); Bosnia and Herzegovina (BH_Ljub1, Ljub2, Mo, GBla, Crn, Bri, Neum, Hut, Sto, Ljubi, Zak, Tre).

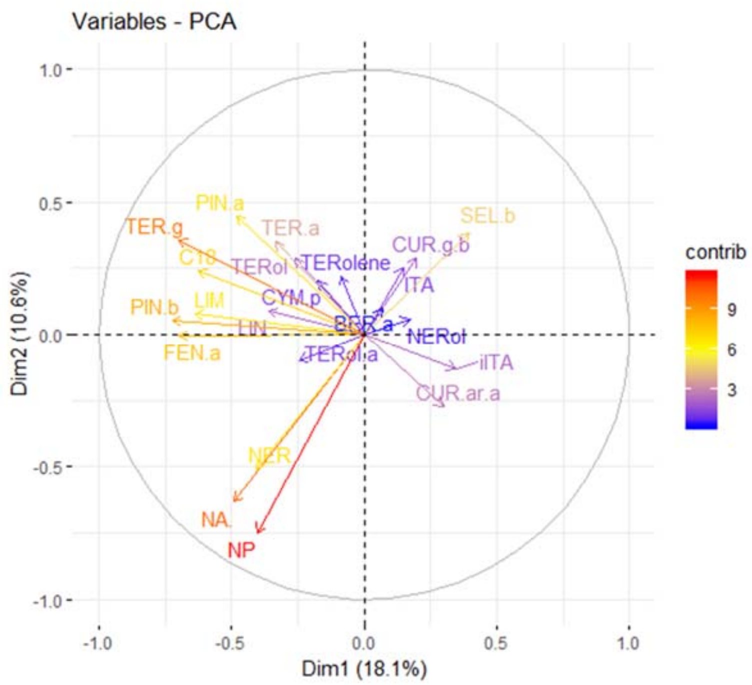


**FIGURE 2**

**The plot of variables of the first two principal components. The importance of variables to the principal components was represented by color gradient of the labels.**

diketone in the essential oils studied here. According to the proportions of individual components in EO, B&H oil can be characterized as α-pinene, neryl acetate, *γ*-curcumene i *β*-diketones type. Analyses of essential oils in different parts of the plant (leaves, flowers, stems) and in different vegetation periods were studied previously [4], therefore they are not the subject of this research. During the flowering period, a higher content of hydrocarbon terpenes than oxygenated terpenes was detected. More neryl acetate was noted in leaves, and *β*-diketone in flowers. Hydrocarbon terpenes were the dominant compounds throughout the year. The highest content of *β*-diketone was recorded in winter, after which it decreased to the lowest values in autumn. Profiles of immortelle essential oils from other Mediterranean areas were also published earlier. It is known that geographical, climatic and other environmental factors influence the composition and amount terpene compounds in immortelle EO. Croatian EOs are categorized as α-pinene, neryl acetate and *γ*-curcumene type [13,14,15,16]. Corsican (France) [3,17,18] and Tuscany (Italy) [19,20] EOs abound in neryl acetates, α-pinene and diketones, whereas EOs from southern regions of Italy have *α*- and *β*-selinene, *γ* -curcumene, carveol, neryl acetate and nerol as dominant components[3]. Montenegro's EO contains mostly neryl acetate and *γ* –curcumene [2]. The Greek EO contains the most geraniol and geranyl acetate [21], and Algerian EO α-cedrene, ar-curumene and geranyl acetae [22]. Data on the essential oils from other regions that used for the comparative study are given in Table 3.

**Principal components analysis.** PCA analysis was performed in order to reveal the relationship between immortelle essential oils and chemical compounds. The first three principal components explained 18.1%, 10.6% and 9.2% of the total variance. The position of oils on the first two principal components (PC) was given in Figure 1. The oils with similar chemical profile are grouped together. The first principal component separated the Italian oils from wild grown immortelle collection of Croatian oils and B&H oils, Elba4 and Elba7 from the rest of Italian oils, Elba and Corsica oils. The group of B&H and Croatian oils positioned close to the center of the plot had low importance to the principal components.

The coordinates of the variables to principal components (Figure 2 and Table 4) explained the differences between the oils. The variables with highest contribution to first principal component were 1,8-cineole, *α*-fenchene, limonene, *β*-pinene and *γ*-terpinene, that were mostly monoterpenes. The highest contribution to the second principal component had oxygenated monoterpenes: neryl acetate, nerol, neryl propionate. The highest contribution to the third principal component had sesquiterpenes: *α*-curcumene, *p*-cymene, isoitalicene, italicene and *β*-selinene. The other compounds had low importance for the principal components.

**TABLE 4**
**The contribution of 22 variables to the first three principal components for 74 essential oils of *Helichrysum italicum*.**

| Compound | PC1 | PC2 | PC3 |
|---|---|---|---|
| *α*-Bergamotene | 0.11 | 0.45 | 2.62 |
| 1,8-Cineole | 9.86 | 2.47 | 2.74 |
| *α*-Curcumene | 2.25 | 3.22 | 12.29 |
| *γ*-, *δ*-Curcumene | 0.98 | 3.57 | 6.89 |
| *p*-Cymene | 0.76 | 1.77 | 15.21 |
| *α*-Fenchene | 12.30 | 0.00 | 0.13 |
| Isoitalicene | 3.02 | 0.75 | 17.65 |
| Italicene | 0.54 | 2.78 | 17.53 |
| Limonene | 10.28 | 0.26 | 3.19 |
| Linalool | 3.26 | 0.33 | 0.07 |
| Neryl acetate | 6.12 | 17.13 | 0.07 |
| Nerol | 4.21 | 11.16 | 0.27 |
| Nerolidol | 0.75 | 0.15 | 0.44 |
| Neryl propionate | 4.11 | 24.15 | 0.14 |
| *α*-Pinene | 5.80 | 8.64 | 2.21 |
| *β*-Pinene | 13.18 | 0.12 | 0.06 |
| *β*-Selinene | 3.87 | 6.22 | 9.70 |
| *α*-Terpinene | 2.84 | 5.24 | 1.60 |
| *γ*-Terpinene | 12.30 | 5.54 | 0.46 |
| Terpinene-4-ol | 1.72 | 3.52 | 2.05 |
| *α*-Terpineol | 1.53 | 0.41 | 1.05 |
| Terpinolene | 0.20 | 2.11 | 3.62 |

The first component contrasted the following compounds: α-pinene, *β*-pinene, *α*-terpinene, *γ*-terpinene, limonene, linalool, 1,8-cineole, *α*-fenchene to *β*-selinene, and the second component contrasted neryl acetate, neryl propionate and nerol to *β*-selinene, *α*-pinene and *γ*-terpinene. That indicated that the first component corresponded to the oils that were high in monoterpenes, linalool and 1,8-cineole but low in *β*-selinene, and the second component contrasted oxygenated monoterpenes to *β*-selinene and monoterpenes.

**Hierarchical clustering on principal components.** The hierarchical clustering was used to reveal grouping of the immortelle oils based on the similarities in chemical composition. Cluster dendrogram grouped the oils in five clusters (Figure 3). Cluster dendrogram showed the distance between clusters. Cluster 2 and 3 were closest and both were close to cluster 1, which were distant to cluster 4 and 5.
Right part of the dendrogram contained most essential oils from B&H, Croatian and Italian collection of wild immortelles that were around Adriatic region, and one oil analyzed from Greece (cluster 2).

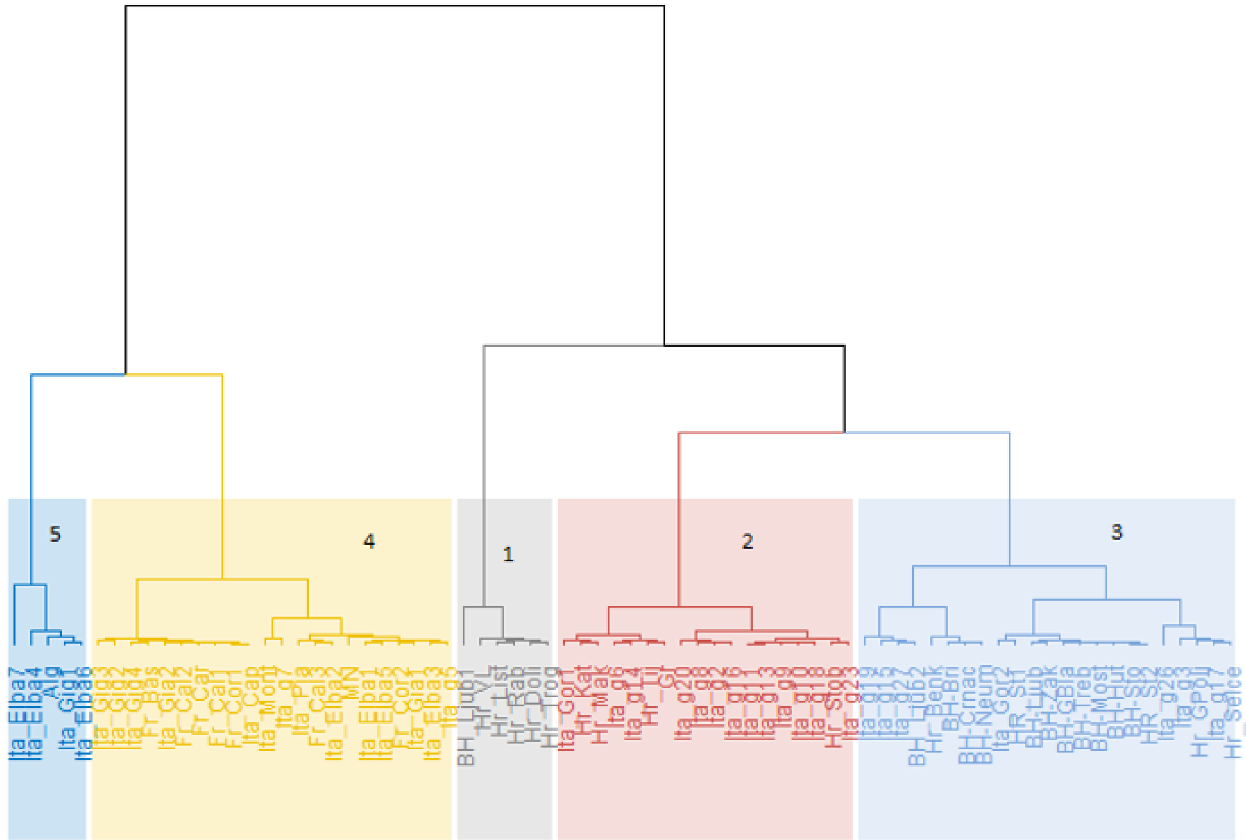


**FIGURE 3**
**Hierarchical analysis of principal components of 74 immortelle essential oils.**
(For sample label see Figure 1.)

The oils from B&H were grouped in cluster 3 (11 oils), with one oil grouped in cluster 1. 14 oils from Croatia, that were analyzed, were divided between cluster 1 (5 oils), cluster 2 (4 oils) and cluster 3 (5 oils). The Italian oils grown on plantations were mostly grouped in cluster 2 (12 oils), while the rest was in cluster 3 (6 oils) and cluster 4 (2 oils). Left part of the dendrogram contained most oils from Tyrrhenian Sea islands: Tuscan archipelago, Corsica and Elba.

Oils from Tuscan archipelago were mostly grouped in cluster 4 (9 oils), while one was in cluster 2 and 5. All oils from Corsica (7 oils) were grouped in cluster 4. Oils from the Elba island were divided between cluster 4 (4 oils) and cluster 5 (3 oils). One oil from Montenegro, which was analyzed, was found in cluster 4 and Algerian oil in cluster 5.

Contribution of compounds to each cluster were given in table 4. Oils from B&H, Croatian and Italian collection on the right part of dendrogram were described mostly by compounds $\alpha$-curcumene, isoitalicene and italicene (cluster 1), $\beta$-salinene and nerol (cluster 2) and $\gamma$- and $\beta$-curcumene, $\alpha$-bergamotene and italicene (cluster 3). On the other hand, oils from Tuscan archipelago, Elba and Corsica on the left part of the dendrogram were described mostly by neryl acetate, neryl propionate and nerol (cluster 4) and $\gamma$-terpinene, $\alpha$-pinene, limonene, $\beta$-pinene and 1,8-cineol (cluster 5). That indicated the difference in chemical composition of oils between clusters.

The oils grouped in clusters were plotted according to the first two principal components (Figure 4). The map confirmed that the oils from Tyrrhenian islands (clusters 4 and 5) were distant from oils from the Adriatic region (clusters 1-3). The map showed the overlapping of the clusters 2 and 3 indicating the similarity in chemical composition of the oils. The hierarchical clustering on principal components was better than PCA for grouping the immortelle oils of the Mediterranean.

## CONCLUSION

The immortelle essential oils collected from wild growing plants in B&H contained 75 % monoterpenes and sesquiterpenes, the rest were diketones, non-terpene esters and non-terpene ketones. The main components of essential oils from this area are $\alpha$-pinene, neryl acetate, $\gamma$-curcumene and $\beta$-diketones. Statistical analyses (PCA and CA) of essential oils from B&H and 64 essential oils from other regions of the Mediterranean indicate that the essential oils from B&H are similar to those from the

Adriatic region (Croatia and southeast Italy) in their chemical composition. The essential oils from Tyrrhenian islands were different from B&H oils. The oils from B&H were distinguished by the content of $\gamma$- and $\beta$-curcumene, $\alpha$-bergamotene and italicene.

The fact of knowing the chemodiversity of the essential oil of this medicinally and economically important plant can facilitate its protection and sustainable management.

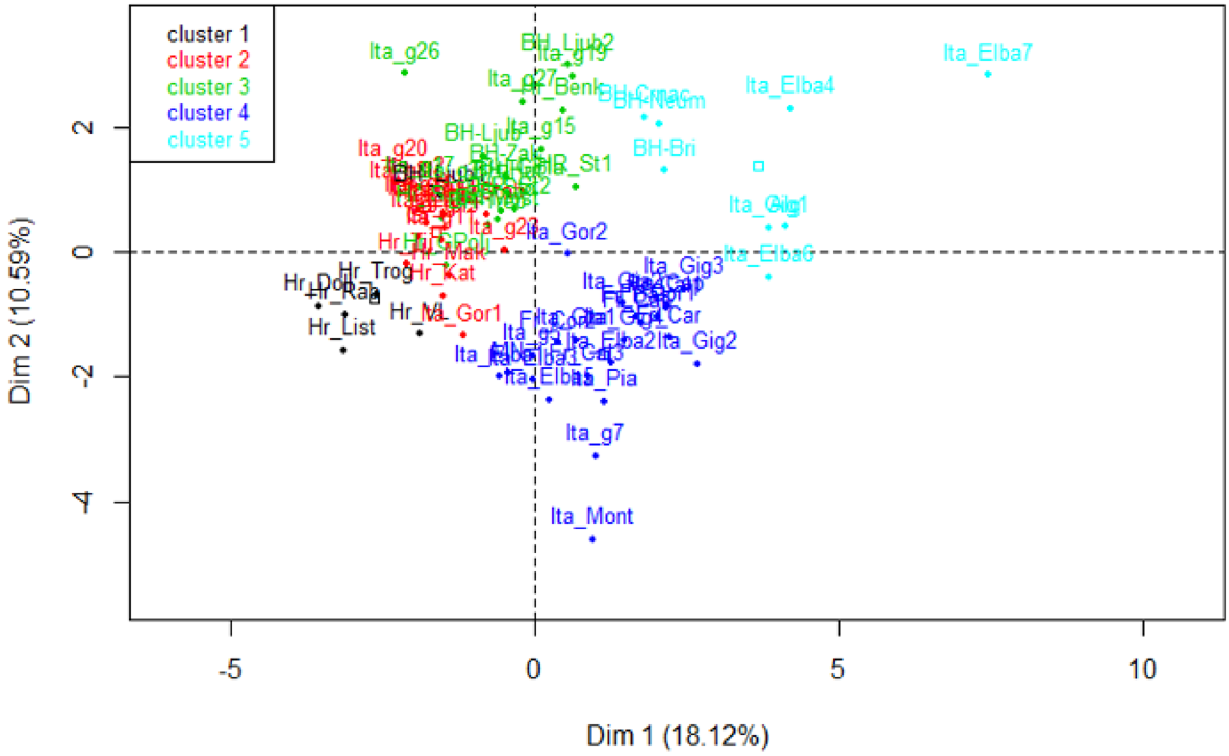


**FIGURE 4**

**Map of the 74 immortelle essential oils according to the principal components.**

(For sample label see Figure 1.)

**TABLE 5**

**The variables that describe the most each cluster.**

| Cluster | Variables | Mean | | Standard deviation | |
|---|---|---|---|---|---|
| | | In category | Overall | In category | Overall |
| 1 | *ar*-, $\alpha$- curcumene | 18.67 | 3.76 | 7.83 | 5.73 |
| | isoitalicene | 5.78 | 0.84 | 3.11 | 1.91 |
| | italicene | 2.92 | 1.65 | 2.90 | 1.40 |
| 2 | $\beta$-salicene | 14.52 | 5.88 | 12.50 | 8.51 |
| | nerol | 1.51 | 2.72 | 0.85 | 2.81 |
| 3 | $\gamma$-, $\beta$-curcumene | 15.31 | 9.41 | 8.85 | 7.10 |
| | $\alpha$-bergamotene | 1.72 | 0.87 | 2.37 | 1.38 |
| | italicene | 2.48 | 1.65 | 1.27 | 1.40 |
| 4 | neryl acetate | 32.83 | 15.64 | 8.72 | 13.52 |
| | neryl propionate | 6.41 | 3.09 | 2.65 | 2.94 |
| | nerol | 4.51 | 2.72 | 3.56 | 2.81 |
| | $\alpha$-fenchene | 0.33 | 0.21 | 0.26 | 0.31 |
| 5 | $\gamma$-terpinene | 1.21 | 0.34 | 0.51 | 0.42 |
| | $\alpha$-pinene | 15.91 | 4.91 | 8.10 | 7.05 |
| | limonene | 6.47 | 3.51 | 2.73 | 2.27 |
| | $\beta$-pinene | 0.71 | 0.31 | 0.61 | 0.31 |
| | 1,8-cineole | 2.84 | 0.68 | 4.08 | 1.72 |
| | linelool | 3.62 | 1.82 | 1.71 | 1.47 |
| | $\alpha$-terpinene | 0.27 | 0.12 | 0.26 | 0.14 |
| | $\alpha$-fenchene | 0.54 | 0.21 | 0.63 | 0.31 |

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