# The Essential Oil of *Bupleurum fruticosum* L. from Corsica: A Comprehensive Study

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A detailed analysis of *Bupleurum fruticosum* oil was carried out by combination of GC (*RI*), GC/MS, and $^{13}$C-NMR analyses. After fractionation by column chromatography, 34 components accounting for 97.8% of the oils were identified. The main component was $\beta$-phellandrene (67.7%), followed by sabinene (9.3%), and limonene (5.6%). The evolution of the chemical composition according to the stages of development of the plant was investigated as well as the composition of leaf, twig, and flower oils. A solvent-free microwave extraction (SFME) of aerial parts was carried out and the composition of the extract compared with that of the essential oil. Finally, 57 oil samples isolated from aerial parts of individual plants, collected all around Corsica, were analyzed, and the data were submitted to statistical analysis. Although the contents of the main components varied, only one group emerged, accompanied with some atypical compositions.

**Introduction.** – *Bupleurum* is a genus of the Apiaceae family comprising *ca.* 200 species and primarily located in the Northern hemisphere, Eurasia, and North Africa. In the Mediterranean basin and North Africa, *ca.* 25 species are represented with some restricted endemism (*B. dianthifolium*, Italy; *B. barceloi* and *B. bourgaei*, Balearic Islands, Spain). About 50 species from this genus *(e.g.*, *B. falcatum*, *B. chinense*, *B. fruticosum*, *etc.*) have been studied chemically, and more than 100 compounds have been isolated (saikosaponins, phenylpropanoids, lignans, coumarins, flavonoids, sterols, *etc.*) [1].

*Bupleurum fruticosum* L. is an evergreen, glaucous, perennial, bushy shrub, 1-to-2-m high, with simple elliptic-oblong leaves, which is represented all around the Mediterranean basin and in the islands [1–3]. *B. fruticosum* emits a strong odor and is repulsive for animals. On the contrary, the fragrant and tasty fruits have been used as spices [4].

Only a few studies were concerned with the chemical composition of the essential oil from *Bupleurum fruticosum.* The first study dates back to 1911 and reported the occurrence, in oil from Sardinia (Italy), of $\alpha$-pinene, $\beta$-phellandrene, and the so-called buplerol, a monoterpene alcohol never identified later in the *B. fruticosum* oils [5]. The next study was reported in 1970 by *Peyron* and *Roubeau* [2]. They identified various monocyclic and bicyclic monoterpenes in an oil sample isolated from the flower heads of plants harvested in the French Alpilles.

Since 1987, various investigations on the chemical composition of *B. fruticosum* essential oil were conducted using modern analytical techniques (*e.g.*, GC/MS in combination with GC retention indices (*RI*)). Although they related to wild growing

and cultivated plants as well as different parts of the plant (aerial parts, leaves, stems, flowers, or fruits), the results may be summarized by the occurrence of four chemical compositions, all dominated by monoterpene hydrocarbons.

The composition dominated by $\beta$-phellandrene was observed in oil samples of various origins: *i*) wild plants from Sardinia, Italy (64.5%; sabinene, 20.7%; aerial parts, leaves, stems, and flowers) [6], *ii*) *in vitro* multiplied plants in the Botanical Garden of the University of Urbino, Italy, (60.9%; sabinene, 12.8%; leaves) [7], and *iii*) wild plants of Eastern Libya (49.3%; $\alpha$-pinene, 15.3%; epigean parts) [8]. Otherwise, $\alpha$-pinene and $\beta$-pinene (41.2 and 35.9%, resp.) were the major components of fruit oil from Spain [9].

Finally, it should be mentioned that oils with different compositions were isolated from leaves and stems of the same plant cultivated at the *Istituto Botanico di Urbino*, Italy. Leaf oil was dominated by $\beta$-phellandrene and sabinene (41.7 and 35.8%, resp. [7], or 38.7 and 39.7%, resp. [4]), while stem oil contained mostly $\gamma$-terpinene and $\alpha$-phellandrene (49.8 and 18.3%, resp. [7], or 48.8 and 12.2%, resp. [4]).

An oil sample from Sicily, Italy, isolated by hydrodistillation/simultaneous solvent extraction, contained $\alpha$-pinene (21.7%), $\beta$-phellandrene (21.3%), and $\beta$-pinene (13.2%) as main components [10].

The aim of the present study was to give an overview of the essential oil from *Bupleurum fruticosum* L. growing wild in Corsica. First, we reported on the detailed analysis of a bulk sample, by combination of chromatographic and spectroscopic techniques. Then, we compared the compositions of leaf, stem, and flower oils. We followed the evolution of the chemical composition of the oil from aerial parts according to the stage of development of the shrub (before, during, and after flowering). Finally, we examined the chemical variability. An interesting point, in our opinion, is that sampling, preparation of the oils, and analyses were carried out at two periods separated by ten years, *i.e.*, in 1997 and 2007. Individual components were identified by combination of GC (*RI*), GC/MS, and $^{13}$C-NMR.

**Results and Discussion.** – *Detailed Analysis of an Oil Sample of* Bupleurum fruticosum. The essential oil was isolated by vapor distillation of the aerial parts of *Bupleurum fruticosum* by the society *U Mandriolu*. It was analyzed by combination of GC (*RI*), GC/MS, and $^{13}$C-NMR. To identify more components and to confirm the identification of some compounds, the bulk sample was submitted to column chromatography (CC) and the fractions were analyzed by GC (*RI*) and $^{13}$C-NMR. Moreover, two minor esters, cinnamyl isovalerate and cinnamyl 2-methylbutyrate were identified by comparison of their spectral NMR data with those of authentic samples prepared by esterification of cinnamyl alcohol.

Individual components of the essential oil, their retention indices on apolar and polar columns, as well as their relative percentages and the mode of identification are reported in *Table 1*. In total, 34 components, which represented 97.8% of the total amount of the oil, were identified, most of them (25) by GC (*RI*), GC/MS, and $^{13}$C-NMR. Among the identified compounds, 18 were hydrocarbons, and the oil was characterized by a high content of $\beta$-phellandrene (67.7%). Two monoterpenes, sabinene (9.3%) and limonene (5.6%), were present in an appreciable amount. A few acyclic, non-terpenic compounds (decan-1-ol, hexyl acetate, octyl acetate, and hexyl

Table 1. *Components of the Essential Oil Isolated from Aerial Parts of* Bupleurum fruticosum L. *from Corsica*

| Components[a]) | Retention index (*RI*) | | Composition [%] | Identification mode |
|---|---|---|---|---|
| | *BP-1* | *BP-20* | | |
| $\alpha$-Thujene | 923 | 1027 | 0.1 | *RI*, MS |
| $\alpha$-Pinene | 931 | 1027 | 1.6 | *RI*, MS, $^{13}$C-NMR |
| Camphene | 944 | 1073 | 0.1 | *RI*, MS |
| Sabinene | 967 | 1129 | 9.3 | *RI*, MS, $^{13}$C-NMR |
| $\beta$-Pinene | 972 | 1116 | 0.3 | *RI*, MS |
| Myrcene | 982 | 1166 | 2.3 | *RI*, MS, $^{13}$C-NMR |
| Hexyl acetate | 994 | 1275 | 0.1 | *RI*, MS, $^{13}$C-NMR |
| $\alpha$-Phellandrene | 999 | 1171 | 2.4 | *RI*, MS, $^{13}$C-NMR |
| $\delta$-Car-3-ene | 1006 | 1153 | 0.4 | *RI*, MS, $^{13}$C-NMR |
| $\alpha$-Terpinene | 1011 | 1176 | 0.2 | *RI*, MS |
| *p*-Cymene | 1013 | 1276 | 0.3 | *RI*, MS, $^{13}$C-NMR |
| Limonene[b]) | 1026 | 1207 | 5.6 | *RI*, MS, $^{13}$C-NMR |
| $\beta$-Phellandrene[b]) | 1026 | 1222 | 67.7 | *RI*, MS, $^{13}$C-NMR |
| $\gamma$-Terpinene | 1050 | 1250 | 1.2 | *RI*, MS, $^{13}$C-NMR |
| Terpinolene | 1080 | 1288 | 0.2 | *RI*, MS |
| Linalool | 1084 | 1550 | 0.1 | *RI*, MS, $^{13}$C-NMR |
| Cryptone | 1157 | 1683 | 0.1 | *RI*, MS, $^{13}$C-NMR |
| Terpinen-4-ol | 1163 | 1605 | 0.3 | *RI*, MS, $^{13}$C-NMR |
| Estragole | 1176 | 1678 | 0.5 | *RI*, MS, $^{13}$C-NMR |
| Octyl acetate | 1193 | 1478 | 0.5 | *RI*, MS, $^{13}$C-NMR |
| Hexyl isovalerate | 1226 | 1448 | 0.2 | *RI*, MS, $^{13}$C-NMR |
| Decanol | 1255 | 1768 | 0.8 | *RI*, MS, $^{13}$C-NMR |
| Bornyl acetate | 1271 | 1583 | tr[c]) | *RI*, MS, $^{13}$C-NMR |
| Citronellyl acetate | 1335 | 1666 | 0.3 | *RI*, MS, $^{13}$C-NMR |
| Geranyl acetate | 1361 | 1762 | 1.9 | *RI*, MS, $^{13}$C-NMR |
| (*E*)-$\beta$-Farnesene | 1448 | 1669 | tr[c]) | *RI*, MS, $^{13}$C-NMR |
| Germacrene D | 1479 | 1713 | tr[c]) | *RI*, MS, $^{13}$C-NMR |
| Bicyclogermacrene | 1495 | 1738 | 0.2 | *RI*, MS, $^{13}$C-NMR |
| $\beta$-Bisabolene | 1503 | 1730 | tr[c]) | *RI*, MS, $^{13}$C-NMR |
| Elemicine | 1517 | 2237 | 0.1 | *RI*, MS, $^{13}$C-NMR |
| Viridiflorol | 1587 | 2093 | 0.3 | *RI*, MS, $^{13}$C-NMR |
| Ledol | 1598 | 2037 | 0.1 | *RI*, MS, $^{13}$C-NMR |
| Cinnamyl 2-methylbutyrate | 1646 | 2310 | 0.3 | *RI*, MS, $^{13}$C-NMR |
| Cinnamyl isovalerate | 1655 | 2334 | 0.4 | *RI*, MS, $^{13}$C-NMR |
| Total | | | 97.8 | |

[a]) Order of elution and percentages of components are given for the apolar column (*BP-1*), except for compounds with [b]). [b]) Percentages are given for the polar column (*BP-20*). [c]) tr: traces.

isovalerate) were also identified. Phenyl propanoids were represented by cinnamyl esters, estragole, and elemicine (*Fig. 1*).

The actual content of $\beta$-phellandrene was determined using quantitative $^{13}$C-NMR spectroscopy. Waiting a period of 5 $T_1$ of the longest $T_1$ value before applying another pulse, combined with a 90° pulse angle, allows both maximal steady-state magnetization and complete relaxation of each C-atom [11]. Applying the aforementioned

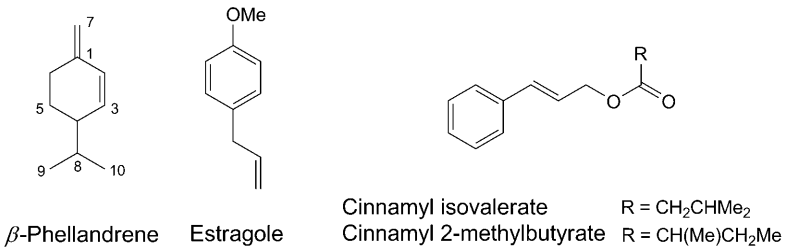


Fig. 1. *Structures of some compounds present in the essential oil of* Bupleurum fruticosum

parameters with the gated-decoupling technique, which provides the suppression of the nuclear *Overhauser* enhancement (NOE), is well-known as the standard sequence for quantitative NMR measurements [12]. The mean value of the integrals of the signals of the protonated C-atoms was compared to those of the $CH_2$ groups of diglyme, used as internal standard (see *Exper. Part*). The percentage of $\beta$-phellandrene determined by GC (FID) is in agreement with that evaluated from NMR data (67.7 and 65.8%, resp.).

*Essential Oils from Various Parts of the Plant.* It could happen that essential oils isolated from different parts of the plant have different compositions. To verify this point, we first compared the composition of leaf and stem oils isolated from two individual plants (*Samples A* and *B*), and we observed, in both cases, that the oils exhibited a very similar composition, the yield of the leaf oil (2.1 and 3.6%) being higher than that of the stem oil (1.6 and 2.1%). More interesting is the comparison of leaf and stem oils with flower oil. The yield of oil isolated from leaves and stems (1.6 and 2.2%) is higher than that isolated from flowers (1.1 and 1.3%). The nine major components of the corresponding oils, identified by GC (*RI*) and $^{13}$C-NMR, accounted for 95.9–97.9% of the total amount (*Table 2*). Although the composition is quite similar with respect to the monoterpene hydrocarbons and oxygenated monoterpenes,

Table 2. *Chemical Composition* (major components) [%] *of the Flower and the Leaf and Stem Essential Oils of* Bupleurum fruticosum L. *from Corsica*

| Components[a]) | *Sample A* | | *Sample B* | |
|---|---|---|---|---|
| | Flower | Leaf and stem | Flower | Leaf and stem |
| $\alpha$-Pinene | 1.5 | 2.0 | 1.6 | 1.9 |
| Sabinene | 2.5 | 1.5 | 1.0 | 1.1 |
| Myrcene | 2.0 | 2.3 | 2.0 | 2.3 |
| $\alpha$-Phellandrene | 3.0 | 3.4 | 3.0 | 3.3 |
| Limonene[b]) | 5.5 | 5.7 | 5.5 | 6.0 |
| $\beta$-Phellandrene[b]) | 71.3 | 77.3 | 71.8 | 79.1 |
| Estragole | 4.8 | – | 6.6 | tr[c]) |
| Decanol | 1.1 | 1.2 | 1.3 | 0.7 |
| Geranyl acetate | 2.3 | 2.4 | 1.7 | 1.2 |
| Yield [% (*w*/*w*)] | 1.1 | 1.6 | 1.3 | 2.2 |

[a]) Order of elution and percentages of components are given for the apolar column (*BP-1*), except for compounds with [b]). [b]) Percentages are given for the polar column (*BP-20*). [c]) tr: traces.

some differences are observed for phenyl propanoids. Indeed, it could be pointed out that the flower oil contained appreciable amounts of estragole (6.6 and 4.8%), not present in leaf and stem oils.

*Solvent-Free Microwave Extraction*. Solvent-free microwave extraction (SFME) is an original combination of microwaves and dry distillation [13]. The essential oils from two individual samples (*Samples C* and *D*), extracted by SFME for 30 min, exhibited a chemical composition qualitatively and quantitatively similar to those obtained by conventional hydrodistillation with a *Clevenger* apparatus for 3–4 h (*Table 3*). We just noted that the content of the major component, $\beta$-phellandrene, was slightly lower, and that higher percentages of oxygenated compounds were observed by SFME in comparison with conventional hydrodistillation. In terms of time and energy, SFME allows, in general, substantial savings of costs and may be considered as a green technology. However, due to the very low yields (0.21 and 0.25%) obtained by this technique in comparison to those attained with conventional hydrodistillation (1.71 and 1.86%), SFME seems not appropriate for the industrial isolation of the volatiles of *Bupleurum fruticosum.*

Table 3. *Chemical Composition* (major components) [%] *of the Essential Oil Isolated from Aerial Parts of* Bupleurum fruticosum *by SFME and by Conventional Hydrodistillation* (HD)

| Components[a]) | *Sample C* | | *Sample D* | |
|---|---|---|---|---|
| | HD | SFME | HD | SFME |
| $\alpha$-Pinene | 2.1 | 1.1 | 2.1 | 1.3 |
| Sabinene | 1.0 | 0.7 | 1.0 | 0.9 |
| $\beta$-Pinene | 0.4 | 0.2 | 0.3 | 0.3 |
| Myrcene | 2.4 | 2.0 | 2.3 | 2.0 |
| $\alpha$-Phellandrene | 3.4 | 3.5 | 3.3 | 2.8 |
| para-Cymene | 0.2 | 0.2 | 0.3 | 0.4 |
| Limonene[b]) | 6.1 | 6.1 | 6.1 | 5.9 |
| $\beta$-Phellandrene[b]) | 79.7 | 76.8 | 79.1 | 77.5 |
| Octyl acetate | 0.3 | 0.5 | 0.3 | 0.5 |
| Decanol | 0.4 | 1.3 | 0.4 | 1.0 |
| Geranyl acetate | 0.8 | 1.2 | 1.6 | 2.5 |
| Elemicin | 0.2 | 0.9 | 0.1 | 0.2 |
| Cinnamyl isovalerate | 0.1 | 0.6 | 0.2 | 0.5 |
| Yield [% (*w*/*w*)] | 1.71 | 0.21 | 1.86 | 0.25 |

[a]) Order of elution and percentages of components are given for the apolar column (*BP-1*), except for compounds with [b]). [b]) Percentages are given for the polar column (*BP-20*).

*Evolution of the Composition before, during, and after Flowering*. The composition of essential oils isolated from aerial parts of plants collected before (May 2005), during (July or September 2005), and after (November 2005) the flowering period are reported in *Table 4*. The 13 major components, which represented from 92.6 to 96.6% of the total amount of the oils, were identified by GC (*RI*) and $^{13}$C-NMR. $\beta$-Phellandrene is strongly predominant all along the physiological cycle of the plant. The percentage of this major component (70.4–78.3%) varied slightly between samples, although the two samples varied in their composition by the content of sabinene (6.0

and 8.2% in *Sample E vs.* 1.2 and 1.5% in *Sample F*). There was no significant variation in the composition of the essential oils collected before, during, and after the flowering period. Conversely, the yield of essential oils varied drastically, being lower (0.8 and 1.2%) before and higher during and after the flowering period (2.4–2.9%) (*Table 4*).

Table 4. *Evolution of the Chemical Composition* (major components) *in* [%] *of the Essential Oils Isolated from Aerial Parts of* Bupleurum fruticosum *before* (BF), *during* (DF), *and after* (AF) *Flowering*

| Components[a]) | *RI* | | *Sample E* | | | *Sample F* | | |
|---|---|---|---|---|---|---|---|---|
| | *BP-1* | *BP-20* | BF | DF | AF | BF | DF | AF |
| $\alpha$-Pinene | 931 | 1027 | 1.9 | 1.8 | 1.8 | 2.0 | 2.0 | 1.9 |
| Sabinene | 967 | 1129 | 7.3 | 6.0 | 8.2 | 1.5 | 1.2 | 1.2 |
| Myrcene | 982 | 1166 | 2.3 | 2.1 | 2.2 | 2.1 | 2.3 | 2.0 |
| Hexyl acetate | 993 | 1273 | 0.2 | 0.1 | 0.2 | 0.1 | 0.5 | 1.2 |
| $\alpha$-Phellandrene | 999 | 1171 | 2.6 | 3.0 | 2.5 | 3.0 | 3.5 | 2.3 |
| *p*-Cymene | 1013 | 1276 | 0.3 | 0.2 | 0.5 | 0.3 | 0.1 | 0.6 |
| Limonene[b]) | 1025 | 1207 | 5.6 | 5.4 | 5.4 | 5.8 | 5.9 | 5.9 |
| $\beta$-Phellandrene[b]) | 1026 | 1222 | 72.8 | 73.6 | 70.4 | 75.7 | 78.3 | 71.6 |
| $\gamma$-Terpinene | 1050 | 1250 | 0.3 | 0.3 | 1.4 | 0.1 | 0.1 | 0.2 |
| Cryptone | 1157 | 1683 | 0.3 | – | 0.2 | 0.8 | – | 2.1 |
| Terpinen-4-ol | 1163 | 1605 | 0.6 | 0.4 | 0.9 | 0.2 | – | 0.1 |
| Decanol | 1255 | 1768 | 0.3 | 1.2 | 0.8 | 1.2 | 0.2 | 1.6 |
| Geranyl acetate | 1361 | 1762 | 1.2 | 1.6 | 2.1 | 1.8 | 1.5 | 1.9 |
| Yield [% (*w*/*w*)] | | | 1.2 | 2.9 | 2.6 | 0.8 | 2.4 | 2.4 |

[a]) Order of elution and percentages of components are given for the apolar column (*BP-1*), except for compounds with [b]). [b]) Percentages are given for the polar column (*BP-20*).

*Chemical Variability.* Aerial parts of 57 individual plants of *Bupleurum fruticosum* were collected in various places in Corsica (*Fig. 2*) from July to September 1997 (20 samples) and from April (or July) to November 2007 (37 samples). The fresh material was hydrodistilled and the oil samples were analyzed by GC (*RI*). Some samples, selected on the basis of their chromatographic profile, were submitted to GC/MS and/or $^{13}$C-NMR analysis. The 57 compositions were submitted to principal component analysis (PCA; *Fig. 3*), and *k*-means partitioning was performed on all the terpene data with individual compounds expressed as a percentage. In PCA, the first two axes accounted for 86.56 and 8.36%, respectively. Only one group emerged, although a few samples, characterized by a lower content of $\beta$-phellandrene and a higher content of sabinene, were present (*Table 5*).

As expected, $\beta$-phellandrene was by far the major component. Indeed, its content varied between 59.0 and 80.3% for all samples, except one (49.1%). Moreover, its content was higher than 70.0% for 72% of the samples. Among other monoterpenes, sabinene accounted for more than 10% in 10 samples out of 57 and reached 29.2% in one sample. Limonene (4.0–9.2%) was always present at appreciable content, while $\alpha$-pinene and $\beta$-pinene reached punctually 8.8 and 6.6%, respectively.

**Conclusions.** – It appears from that study on a large group of samples (57), harvested in various locations of a well-defined area, that *Bupleurum fruticosum*

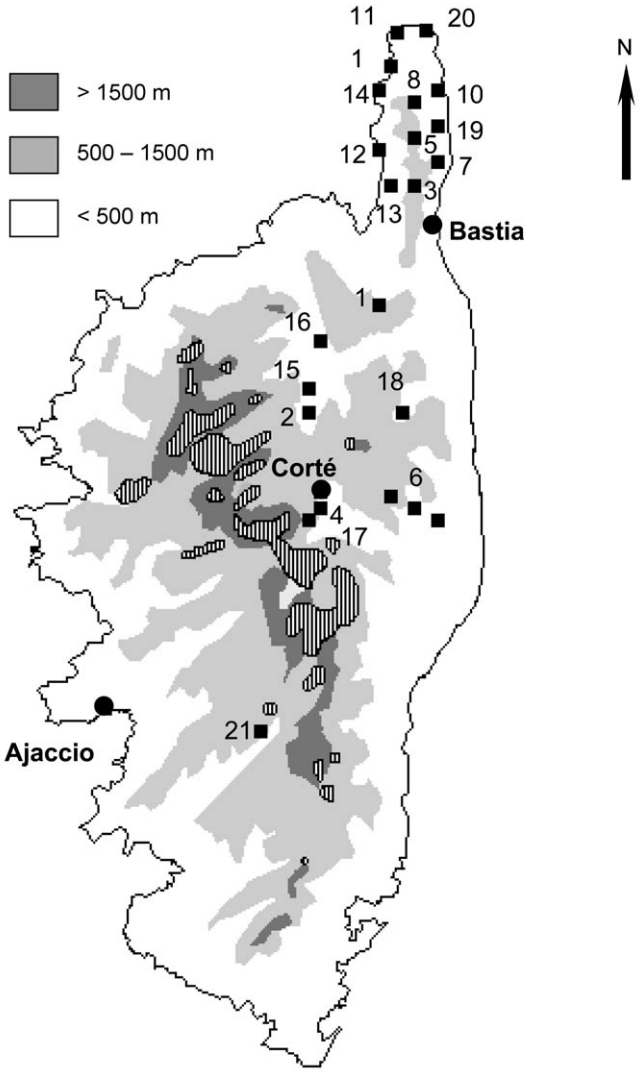


Fig. 2. *Sampling locations of* Bupleurum fruticosum. ●: Main city; ■: Sampling location: 1, Barchetta; 2, Caporalino; 3, Cardo; 4, Casanova de Venaco; 5, Casella; 6, Cateraggio; 7, Erbalunga; 8, Luri; 9, Marinca; 10, Meria; 11, Morsiglia; 12, Nonza; 13, Patrimonio; 14, Pino; 15, Ponte Leccia; 16, Ponte Novo; 17, Saint-Pierre de Venaco; 18, San Nicolau; 19, Sisco; 20, Tolare; 21, Tolla.

growing wild in the island of Corsica produces a $\beta$-phellandrene-rich oil (more than 70% for 72% of the samples). For comparison purposes, it could be noted that the highest percentage of $\beta$-phellandrene reported so far in the literature reached 64.5% (Italy). No sample dominated by $\gamma$-terpinene, $\alpha$-pinene, $\beta$-pinene, or sabinene was detected. Leaves and stems produced oils with similar composition. The composition of flower oil, although dominated by $\beta$-phellandrene, exhibited a small content of the

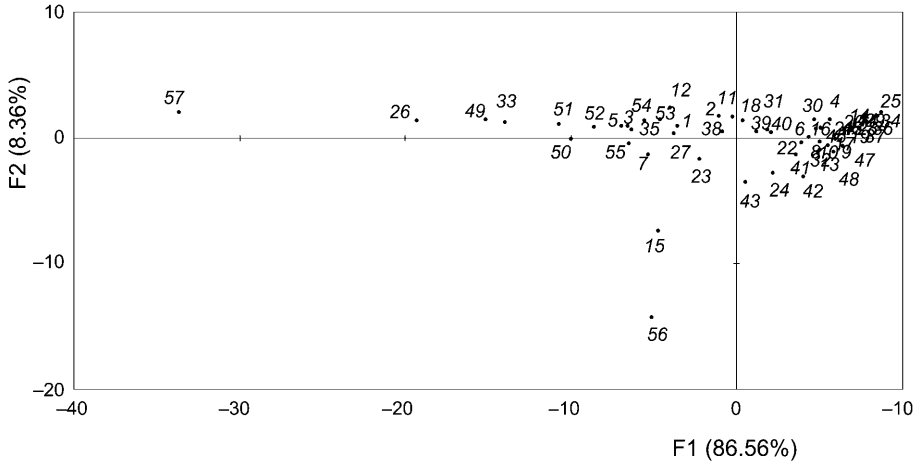


Fig. 3. *Principal component analysis scatter plot of 57 samples of* Bupleurum fruticosum *essential oil from Corsica*

Table 5. *Chemical Variability* (major components) [%] *of 57 Samples of* Bupleurum fruticosum *Essential Oil.* Results are given as mean value (Mean) with standard deviation (SD), minimum content (Min), and maximum content (Max).

| Components[a]) | Mean | SD | Min | Max |
|---|---|---|---|---|
| $\alpha$-Pinene | 2.1 | 1.18 | 1.6 | 8.8 |
| Sabinene | 5.3 | 5.67 | 0.6 | 29.2 |
| $\beta$-Pinene | 0.5 | 1.03 | 0.3 | 6.6 |
| Myrcene | 1.5 | 0.88 | 1.8 | 2.5 |
| $\alpha$-Phellandrene | 2.6 | 0.49 | 1.7 | 3.6 |
| *p*-Cymene | 1.1 | 1.09 | 0.1 | 1.1 |
| Limonene[b]) | 5.6 | 0.63 | 4.0 | 9.2 |
| $\beta$-Phellandrene[b]) | 72.5 | 6.09 | 49.1 | 80.3 |
| $\gamma$-Terpinene | 0.6 | 0.73 | tr[c]) | 3.1 |
| Cryptone | 0.4 | 0.48 | – | 3.1 |
| Terpinen-4-ol | 0.6 | 0.57 | tr[c]) | 2.1 |
| Estragole | 0.4 | 0.32 | 0 | 1.7 |
| Decanol | 1.0 | 0.50 | 0.2 | 2.5 |
| Geranyl acetate | 1.6 | 0.45 | 0.6 | 2.5 |

[a]) Order of elution and percentages of components are given for the apolar column (*BP-1*), except for compounds with [b]). [b]) Percentages are given for the polar column (*BP-20*). [c]) tr: traces.

phenylpropanoid estragole (*Fig. 1*). The composition of oils extracted from aerial parts varied only slightly, qualitatively and quantitatively, along the physiological cycle of the plant. However, the yield was higher during and after the flowering stage. Finally, oil produced by SFME had a similar composition than that isolated by conventional hydrodistillation, but the yield was drastically lower.

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## Experimental Part

*Plant Material.* To differentiate oils isolated from various parts of the plant, leaves, stems, and flowers of *Bupleurum fruticosum* L. were collected from two plants growing wild near Corte, in the centre of Corsica, in September 2005, and they were submitted independently to hydrodistillation. Aerial parts of two individual plants were collected in May, in July or September, and in November 2005 to follow the evolution of the composition before, during, and after flowering. Finally, aerial parts of individual plants were collected from July to September 1997 (20 samples) and 2007 (37 samples), in the following locations: Barchetta, Caporalino, Cardo, Casanova de Venaco, Casella, Cateraggio, Erbalunga, Luri, Marinca, Meria, Morsiglia, Nonza, Patrimonio, Pino, Ponte Leccia, Ponte Novo, Saint-Pierre de Venaco, San Nicolau, Sisco, Tolare, and Tolla (*Fig. 2*). It could be noted that *B. fruticosum* is abundant in the so-called 'Alpine Corsica' and it is scarce in the other part, called 'Crystalline Corsica' [14]. Identification of the species was confirmed by Mrs. *Laetitia Hugot*, *Office de l'Environnement de la Corse.*

*Essential-Oil Isolation and Fractionation.* The oil sample used for detailed analysis was supplied by the society *U Mandriolu*, Corsica. It was obtained by vapor distillation in an industrial apparatus, with plants harvested during July 2005, near Corte. The bulk sample (1.030 g) was submitted to column chromatography (CC) on silica gel ($SiO_2$). Elution with a solvent gradient of increasing polarity (pentane/$Et_2O$ 100:0–0:100) led to four fractions: *Fr. 1* (812 mg) and *Fr. 2* (18 mg) eluted with pentane, *Fr. 3* (115 mg) eluted with pentane/$Et_2O$ 97:3, and *Fr. 4* (86 mg) eluted with $Et_2O$.

Otherwise, the fresh material (aerial parts or various organs of the plant) was submitted to hydrodistillation for 3 h, using a *Clevenger*-type apparatus. The yield of oil was expressed as % (*w*/*w*) *vs.* fresh material. Solvent-free microwave extraction (SFME) was carried out using a *Milestone DryDist* microwave apparatus. The temp. was monitored and controlled by feedback to the microwave power regulator. Extraction time, 30 min; power, 500 W.

*Analytical GC.* GC Analysis was carried out with a *Perkin-Elmer Autosystem* GC apparatus equipped with FID and two fused-silica cap. columns (50 m × 0.22 mm i.d., film thickness 0.25 μm), *BP-1* (poly(dimethylsiloxane)) and *BP-20* (poly(ethyleneglycol)). The oven temp. was programmed from 60 to 220° at 2°/min and then held isothermal at 220° for 20 min; injector temp., 250°; detector temp., 250°; carrier gas, He (0.8 ml/min); split, 1:60. The relative proportions of the oil constituents were expressed as percentage obtained by peak area normalization, without using correcting factors. Retention indices (*RI*) were determined rel. to the retention times of a series of *n*-alkanes with linear interpolation (*Target Compounds* software from *Perkin-Elmer*).

*GC/MS Analysis.* The essential oils were analyzed with a *Perkin-Elmer TurboMass* detector (quadrupole), directly coupled to a *Perkin-Elmer Autosystem XL*, equipped with a fused-silica cap. column (60 m × 0.22 mm i.d., film thickness 0.25 μm), *Rtx-1* (polydimethyl siloxane). Carrier gas, He (1 ml/min); split, 1:80; injection vol., 0.2 μl; injector temp., 250°; oven temp. programmed from 60° to 230° at 2°/min and then held isothermal at 230° for 45 min; ion-source temp., 150°; energy ionization, 70 eV; electron ionization mass spectra were acquired over the mass range 35–350 Da.

*NMR Analysis.* All $^{13}$C-NMR spectra were recorded in $CDCl_3$, with chemical shifts referred to int. $Me_4Si$. Spectra of mixtures (essential oil or CC fractions) isolated in 2007 were recorded on a *Bruker AVANCE 400 Fourier Transform* spectrometer, operating at 100.63 MHz, equipped with a 5-mm probe, with the following parameters: pulse width, 4 μs (flip angle 45°); acquisition time, 2.7 s for 128 K data table with a spectral width of 24000 Hz (240 ppm); CPD mode decoupling; digital resolution, 0.183 Hz/pt. The number of accumulated scans was 3000 for each sample (*ca.* 40 mg of the sample in 0.5 ml of $CDCl_3$). Spectra of oil samples isolated in 1997 were recorded on a *Bruker AC 200 Fourier Transform* spectrometer operating at 50.323 MHz, equipped with a 10-mm probe, with the following parameters: pulse width, 5.0 μs (flip angle 45°); spectral width, 12500 Hz (250 ppm); CPD mode decoupling. The

number of accumulated scans was 3000–5000 for each sample (200 mg of the oil in 2 ml of $CDCl_3$). An exponential multiplication of the free induction decay with line broadening of 1.0 Hz was applied before *Fourier* transformation.

$^1$H-, $^{13}$C-, and 2D-NMR Spectra (DEPT, HSQC, HMBC) of pure compounds were recorded using *Bruker* microprograms (*Bruker AVANCE 400*). For $T_1$ measurements, the longitudinal relaxation delays of the $^{13}$C-nuclei ($T_1$ values) were determined for each protonated C-atom of $\beta$-phellandrene (*Fig. 1*) and for the two $CH_2$ groups of diglyme (bis(2-methoxyethyl) ether) by the inversion-recovery method, using the standard sequence 180°-$\tau$-90°-$D$1, with a relaxation delay $D$1 of 10–50 s (*Table 6*). Each delay of inversion ($\tau$) was thus taken into account for the computation of the corresponding $T_1$ using *Eqn. 1*.

$$I_P = I_0 + p \cdot e^{-\tau/T_1} \quad (1)$$

Table 6. *$^1$H-, $^{13}$C-, and 2D-NMR Data of $\beta$-Phellandrene.* At 400/100 MHz, respectively, in $CDCl_3$; $\delta$ in ppm, $J$ in Hz.

| Position | $\delta$(C) | $T_1$ [s] | DEPT | $\delta$(H) |
|---|---|---|---|---|
| 1 | 143.72 | n.m.[a]) | C | – |
| 2 | 129.62 | 7.9 | CH | 6.16 (*dd*, $J$=9.8, 2.7) |
| 3 | 134.15 | 7.9 | CH | 5.75 (br. *d*, $J$=9.8) |
| 4 | 42.16 | 7.4 | CH | 2.00–2.10 (*m*) |
| 5 | 25.81 | 4.0 | $CH_2$ | 1.77 (*dq*, $J$=12.7, 4.0), 1.43 (*tdd*, $J$ = 12.7, 10.0, 3.8) |
| 6 | 30.25 | 4.2 | $CH_2$ | 2.44 (*dt*, $J$ = 14.9, 3.8), 2.29 (*dd*, $J$ = 14.9, 12.7) |
| 7 | 109.95 | 4.6 | $CH_2$ | 4.76–4.78 (*m*), 4.73–4.75 (*m*) |
| 8 | 31.99 | 7.5 | CH | 1.60–1.70 (*m*) |
| 9 | 19.54 | 3.5 | $CH_3$ | 0.94 (*d*, $J$=6.7) |
| 10 | 19.73 | 3.5 | $CH_3$ | 0.91 (*d*, $J$=6.7) |

[a]) n.m.: Not measured

For the quant. determination of $\beta$-phellandrene in the oil sample of the society *U Mandriolu*, a quantitative spectrum was acquired using the inverse gated decoupling sequence with the following parameters: pulse width, 8.5 µs (flip angle 90°); acquisition time, 2.7 s for 128 K data table with a spectral width of 24000 Hz (240 ppm); repetition time 40 s (5 × longest $T_1$ of protonated C-atoms, *Table 6*); digital resolution, 0.183 Hz/pt. The number of accumulated scans was 256 (52 mg of the oil sample in 0.5 ml of $CDCl_3$). Diglyme was used as int. standard ($T_1$ of $CH_2$ group C-atoms, 3.8 s). The amount, $m_P$ in mg, of $\beta$-phellandrene was determined using *Eqn. 2:*

$$m_P = \frac{2A_P \cdot M_P \cdot m_D}{A_D \cdot M_D} \quad (2)$$

$A_P$, mean values of the integrals of the protonated C-atoms of $\beta$-phellandrene; $A_D$, mean values of the integrals of the two $CH_2$ groups of diglyme used as int. standard; $M_P$ and $M_D$, molecular weight [g/mol] of $\beta$-phellandrene and diglyme, resp.; $m_D$, weight of diglyme [mg]. Experimental data: $A_P$, 1.711; $A_D$, 1.006; $M_P$, 136.23 g/mol; $M_D$, 134.17 g/mol; $m_D$, 10.0 mg; $m_{EO}$, 52.0 mg; $m_P$, 34.2; $m_P$% = 65.8.

*Identification of Components.* Identification of the individual components was based: *i*) on comparison of their GC retention indices (*RI*) on polar and apolar columns with those of authentic compounds and literature data [15][16], *ii*) on computer matching with commercial mass spectral libraries [17–19] and comparison of spectra with literature data [15][20][21], and *iii*) on comparison of the signals in the $^{13}$C-NMR spectra of the mixtures with those of reference spectra compiled in the laboratory spectral library, with the help of a laboratory-made software [22–24]. In the investigated samples, individual components were identified by NMR at contents as low as 0.5%.

*Preparation of Cinnamyl Esters.* To a soln. of cinnamyl alcohol (276 mg, 2.05 mmol or 519 mg, 3.87 mmol, resp.) in 30 ml $CH_2Cl_2$ and $Et_3N$ (326 mg, 3.22 mmol or 780 mg, 7.74 mmol, resp.) cooled to

0°, a soln. of 2-methylbutyryl chloride (518 mg, 4.29 mmol) or isovaleryl chloride (978 mg, 7.74 mmol) in $CH_2Cl_2$ (30 ml) was added dropwise. The mixture was stirred until r.t. was reached and then refluxed for 3 h. The mixture was poured into 200 ml of cold $H_2O$. After decantation, the org. phase was separated, washed twice with $H_2O$, dried ($Na_2SO_4$), and concentrated in vacuum. The crude esters were purified by CC ($SiO_2$). Yields (not optimized) were 80 and 79%, resp.

*Cinnamyl 2-Methylbutyrate.* $^1$H-NMR: 7.37–7.40 (*m*, 2 H); 7.29–7.33 (*m*, 2 H); 7.23–7.26 (*m*, 1 H); 6.65 (*d*, *J* = 15.9, 1 H); 6.28 (*dt*, *J* = 15.9, 6.4, 1 H); 4.74 (*dd*, *J* = 6.4, 1.2, 2 H); 2.43 (*sext.*, *J* = 7.0, 1 H); 1.66–1.77 (*m*, 1 H); 1.44–1.55 (*m*, 1 H); 1.17 (*d*, *J* = 7.0, 3 H); 0.92 (*t*, *J* = 7.4, 3 H). $^{13}$C-NMR: 176.56; 136.32; 133.96; 128.61; 128.02; 126.61; 123.48; 64.79; 41.12; 26.83; 16.64; 11.65.

*Cinnamyl Isovalerate.* $^1$H-NMR: 7.37–7.40 (*m*, 2 H); 7.30–7.35 (*m*, 2 H); 7.25–7.28 (*m*, 1 H); 6.65 (*d*, *J* = 15.8, 1 H); 6.28 (*dt*, *J* = 15.8, 6.4, 1 H); 4.74 (*dd*, *J* = 6.4, 1.4, 2 H); 2.24 (*d*, *J* = 7.3, 2 H); 2.08–2.19 (*m*, 1 H); 0.97 (*d*, *J* = 6.7, 6 H). $^{13}$C-NMR: 172.96; 136.28; 134.08; 128.61; 128.04; 126.61; 123.37; 64.79; 43.44; 25.75; 22.44.

## REFERENCES


[1] S. L. Pan, 'Traditional Herbal Medicines for Modern Times. *Bupleurum* Species – Scientific Evaluation and Clinical Application', Taylor & Francis, New York, 2006.
[2] L. Peyron, M. Roubeau, *Plant. Med. Phytother.* **1970**, *4*, 172.
[3] V. Picci, A. Atzei, A. Manunta, *Rivista Ital. EPPOS*, **1974**, *56*, 239.
[4] A. Manunta, B. Tirillini, D. Fraternale, *J. Essent. Oil Res.* **1992**, *4,* 461.
[5] L. Francesconi, E. Sernagiotto, *Atti R. Acc. Lincei* **1913**, *22*, 34.
[6] A. Manunta, I. Morelli, V. Pizzi, *Plant. Med. Phytother.* **1987**, *21*, 20.
[7] A. Bertoli, L. Pistelli, I. Morelli, D. Fraternale, L. Giamperi, D. Ricci, *Plant Sci.* **2004**, *167*, 807.
[8] L. Giamperi, D. Ricci, D. Fraternale, A. Manunta, R. Tabacchi, *J. Essent. Oil Res.* **1998**, *10*, 369.
[9] I. Lorente, M. A. Ocete, A. Zarzuelo, M. M. Cabo, J. Jimenez, *J. Nat. Prod.* **1989**, *52*, 267.
[10] G. Dugo, A. Trozzi, A.Verzera, A. Rapisarda, *Essenze Derivati Agrumari* **2002**, *70*, 201.
[11] H. Günther, in 'La Spectroscopie de RMN', Masson, Paris, 1994, p. 246.
[12] J. R. Mooney, in 'Analytical NMR', Eds. L. D. Field, S. Sternhell, John Wiley & Sons, Chichester, 1989, p. 45.
[13] M. E. Lucchesi, F. Chemat, J. Smadja, *J. Microwave Power Electromagn. Energ.* **2004**, *39*, 135.
[14] J. Gamisans, D. Jeanmonod, 'La Végétation de la Corse', Edisud, Aix-en-Provence, 1999.
[15] D. Joulain, W. A. König, 'The Atlas of Spectral Data of Sesquiterpene Hydrocarbons', EB-Verlag, Hamburg, 1998.
[16] N. W. Davies, *J. Chromatogr., A* **1990**, *503*, 1.
[17] F. W. McLafferty, D. B. Stauffer, 'Wiley Registry of Mass Spectral Data: Mass Spectrometry Library Search System Bench-Top/PBM, version 3.10d', 6th edn., Palisade, Newfield, 1994.
[18] National Institute of Standards and Technology, 'The NIST/EPA/NIH Mass Spectral Library', PC Version 1.7, Perkin-Elmer Corporation, Norwalk, CT, 1999.
[19] W. A. König, D. H, Hochmuth, D. Joulain, 'Terpenoids and Related Constituents of Essential Oils. Library of MassFinder 2.1', Institute of Organic Chemistry, Hamburg, 2001.
[20] F. W. McLafferty, D. B. Stauffer, 'The Wiley/NBS Registry of Mass Spectral Data', 4th edn., Wiley-Interscience, New York, 1988.
[21] R. P. Adams, 'Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy', 4th edn., Allured, Carol Stream, Illinois, 2007.
[22] F. Tomi, P. Bradesi, A. Bighelli, J. Casanova, *J. Magn. Reson. Anal.* **1995**, *1*, 25.
[23] S. Rezzi, A. Bighelli, V. Castola, J. Casanova, *Appl. Spectrosc.* **2002**, *56*, 312.
[24] F. Tomi, J. Casanova, *Acta Horticult.* **2006**, *723*, 185.


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