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# Contribution to the Analysis of the Volatile Constituents from some Lavender and Lavandin Cultivars Grown in Greece

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The essential oils of Lavandula *angustifolia* and the Lavandin hybrids *"super"* and *"special"* grown in Greece, were studied in order to evaluate their quality as far as the correlation of their essential oil composition. The essential oil yields were determined to 4.4 %, 7.5% and 8.5% in the lavandula and lavandin *"super"* and *"special"* hybrids respectively. Fifty-nine constituents were identified representing the 99% of the oils. The qualitative analysis of the oils was carried out by GC/MS and revealed that linalool and linalylacetate were the predominant constituents. Remarkable differences were noticed between the camphor percentages among them (11.35% and 5.03% for the *"super"* and *"special"* respectively).

**Keywords**: *Lavandula angustifolia*, Lavandin, essential oil yield, GC/MS analysis, linalool, linalylacetate

## Introduction

Interest in growing lavender has taken an upsurge in the past ten years, with the old-fashioned lavender fragrance becoming popular again, perhaps because of its use in aromatherapy[1]. Essential oil of lavender is also used as flavoring for various foods and beverages, though non-food products manufactured with lavender oil include soaps, colognes, and other cosmetics. Lavender may be grown as bedding plant, herb specimen, tea plant, bee forage, or source of fragrance in landscaping designs.

Lavender is a popular aromatic and perennial Mediterranean herb, belonging to Lamiaceae family, growing almost all over the world Asia, America, Australia, and almost all over Europe. It is marked as the fresh or dried plant.

Among the Lavender species, *Lavandula angustifolia* Mill. produces the best quality's essential oil for the industry, and due to the high linalool content, it is used in the aromatherapy for its mild sedative activity as well as in antibacterial and antifungal medicaments [2,3]. It is also reported in the folk medicine[4] because of the carminative, diuretic and stomachic properties. Very high-quality essential oil of lavender is required for use in the alternative health practice of aromatherapy, as far as in pharmaceutics.

Lavandin oils are distilled from hybridized plant *Lavandula hybrida* Reverchon, a cross between *Lavandula angustifolia* Mill. and *Lavandula latifolia* Medic. Lavandin plants are vigorous and extremely productive, though their essential oils yield can be 2-3 times higher compared with *L. angustifolia*[5]. They produce high yields of lower quality and priced lavender oils, used widely in the

production of inexpensive perfumes, soaps, bath products, detergents etc., or in the adulteration of the high priced lavender oils[6]. There are many successful commercial hybrids, the most of France origin, cultivated in many parts of Europe, among them the best known are *"abrialis", "super", and "grosso"*.

In Greece, lavender is growing in many areas and cultivated in several districts but not systematically. Because of the great commercial interest of lavender oils the aim of the present work was to study the essential oil of a) *Lavandula angustifolia* Mill. grown in Northern Greece, though as far it concerns to our knowledge, there are no reported data on the Greek oil and b) the essential oils of two commercial Lavandin hybrids cultivated in Greece, the *"super"* and *"special"*.

## Results and Discussion

The distillation of L. *angustifolia* Mill. and of *super* and *special* Lavandin hybrids yielded pale yellow essential oils, which percentage yields' were determined as 4.4%, 7.5%, and 8.5% respectively. These yields are classified to the highest reported in the literature for Lavender oils[7,8,9]. The yields of the hybrids were significantly higher than those of *L. angustifolia*, the *special* hybrid being more efficient than the *super* one (93% and 70% higher yields, respectively, compared to *L. angustifolia*).

In the qualy-quantitative analysis of the oils, performed by GC and GC-MS, were detected more than eighty constituents and fifty-nine of them were identified, representing at least the 99% of the oils. The percentages reported were based on the data after three chromatographic runs (Srel.± 1.01 - ±1.2%). The constituents were identified by comparing the retention times on three columns of different polarity with those of authentic samples using the peak enrichment technique. For compounds that pure samples were not available, identification was carried out by comparison with references oils and mainly by mass spectrometry, matching the peak mass spectra to those of NIST 98 mass spectra library, and of the commercial library Newterp for essential oils[10].

In Table 1 are listed the identified constituents of lavender, and of *super* and *special* lavandin hybrids respectively. Although the number of components of lavender essential oil resulted high, the main components (>1%) representing the 91.54% of the total sample, were eleven - linalool (50.63%), linalylacetate (15.72%), terpinen-4-ol (7.84%), cis-ocimene (4.25%), trans-ocimene (2.73)%, lavandulyl-acetate (2.73%), β-caryophyllene (2.04%), lavandulol (1.52%), α-terpineol (1,52%), 3-octanone (1.36%) and cryptone (1.2%). They correspond to the most significant compounds as indicated by extensive studies performed on lavender plants[11,12,13].

The percentage amount of linalool (50.63%) is among the highest yields encountered in the literature; 50.52% was recorded for the cultivars Munsteam grown in the United States[12], 49.90% for an Italian Lavender sample oil, 49.86% and 44.44% for different French cultivars[14,15]. Camphor and 1,8 cineol, which undermine the quality of the oil, accounted for 0.06 and 0.66% of the oil respectively. Nevertheless, the amounts of terpinen-4-ol, and the typical components for lavender oil, lavandulol and lavandulylacetate were also remarkable.

The oxygenated compounds nerol, nerylacetate, geranylacetate (known as the "rhodinol" part of the oil) that are important for the overall aroma, donating a sweet and pleasant note to the oil, contributed to the lavender oil as well – 1.76% (Table 2).

The *super* and *special* lavandin hybrids contained linalool as main component (23.01% and 37.69 % respectively), though the linalylacetate content varied from 20.35% to 29.14% falling within the ranges of the typical lavandin oils[14]. Significant differences were observed between the *super* and *special* hybrids concerning the percentages of 1,8 cineol (15.85% and 5.39%), camphor (11.35% and 5.03%), terpinen-4-ol (6.67% and 0.08%) and those of lavandulylacetate (0.37% and 2.2%)

respectively. The amounts of camphor and 1,8 cineol of *super* lavandin essential oil (11.35% and 15.85% respectively) match those noted by Melegari *et al.*[16], for Italian *super* cultivars. In contrast, the camphor and 1,8 cineol were found in lower quantities in the *special* hybrid (5.03 and 5.39% respectively). Moreover the group of the components constituting of the "rhodinol" part was higher in the *special* hybrid: 2.92% versus 2.43% in the *super* one (Table 2), nevertheless both values were higher than those recorded for known Italian lavandin oils[17].
In conclusion, Lavender and Lavandin cultivated in Greece produce essential oils with quite high yields (4.4%, 7.5%, and 8.5%). Moreover the *super* and *special* hybrids show good adaptability to the Greek climate and environmental conditions, the latter producing an essential oil of superior yield and quality.

## Experimental

### Plant material

The plant material was originated from experimental fields established in Northern Greece, full sun exposed, with soil conditions; moderate alkaline PH, well drainage and sufficient in all the nutrients. The fields were not irrigated or fertilised, not even any kind of plant protection was applied.
For analysis, samples of lavender and lavandin plants (flowering tops) were collected in July, from the two middle rows (of four rows) of the experimental fields at full bloom stage. Vouchers of the samples investigated are kept at the Herbarium of the Institute of Aromatic and Medicinal plants, NAGREF.

### Isolation and yield of the essential oils

The percentage oil yield was determined using the European Pharmacopoeia apparatus (Clevenger type) after 3 replications (SD ± 0.3%). The samples of 20g of plant material were distilled for 1 hour and 30 min at distillation rates 3-3.5 ml/min[18]. The oils were dried over anhydrous $Na_2SO_4$ and stored with 3 ml of n-pentane in sealed glass vials under refrigeration (-20$^0$C).

### Gas Liquid Chromatography and Gas Chromatography / Mass Spectrometry

The essential oil samples were analysed by gas chromatography, using a Gas Chromatograph Hewlett Packard 5890 Series II, equipped with one injection port and a two-channel system of columns and respective FIDs, connected to a chromatographic integrator (Hewlett Packard 3396 Series II Dual Channel). Sample injection 0.2 – 0.3 µl of a 10% essential oil solution in pentane; split 1:20. Three different polarity fused silica columns were used: a) Durabond – DB 1, b) DB-Wax both of 60m x 0.25 mm i.d., film thickness 0.25 µm (J & W Scientific Inc., Rancho Cordova, California, USA), and c) CP-Sil 19 CB, 25m x 0.25 mm i.d., film thickness 0.20 µm (Chrompack Nederland, Middelburg, The Netherlands). Oven temperature: 45 to 220$^0$ C at 3.5$^0$ C/min, carrier gas nitrogen at 140 Kpa, injection temperature 220$^0$ C, detectors' temperature 300$^0$ C. The percentage compositions were computed from the GC peak areas without correction factors.
The identification of the constituents was carried out 1) using the peak enrichment technique of reference compounds (authentic samples by Roth, Aldrich and Sigma), 2) with the chromatograms comparison to those of essential oils previously analyzed and published from our data bank and

**Table 1.** Percentage composition of the volatiles constituents from *Lavandula angustifolia* and of the hybrids "*Super*" and "*Special*" cultivated in Greece

| Nr[a] | KI[b] | Constituents | *Lavandula angustifolia* | "*Super*" | "*Special*" | Method of Identification [c] |
|---|---|---|---|---|---|---|
| | | | Percentage yield | | | |
| 1 | 0931 | α-Thujene | 0,19 | 0,11 | - | 1,2,3 |
| 2 | 0939 | α-Pinene | 0,24 | 0,68 | 0,19 | 1,2,3 |
| 3 | 0953 | Camphene | 0,04 | 0,31 | 0,18 | 1,2,3 |
| 4 | 0976 | Sabinene | 0,10 | 0,38 | 0,10 | 1,2,3 |
| 5 | 0978 | 1-Octen-3ol | 0,17 | - | 0,04 | 1,2,3 |
| 6 | 0980 | β-Pinene | 0,05 | 0,95 | 0,21 | 1,2,3 |
| 7 | 0986 | 3-Octanone | 1,36 | - | 0,89 | 1,2,3 |
| 8 | 0991 | β-Myrcene | 0,61 | 0,99 | 0,91 | 1,2,3 |
| 9 | 0993 | 3-Octanol | 0,27 | - | 0,09 | 1,2,3 |
| 10 | 1005 | α-Phellandrene | 0,04 | 0,03 | - | 1,2,3 |
| 11 | 1008 | Hexyl acetate | - | 0,20 | 1,07 | 1,2,3 |
| 12 | 1011 | Δ3-Carene | 0,12 | 0,07 | - | 1,2,3 |
| 13 | 1018 | α-Terpinene | - | 0,08 | - | 1,2,3 |
| 14 | 1026 | p-Cymene | 0,18 | 0,06 | - | 1,2,3 |
| 15 | 1031 | Limonene | 0,89 | - | - | 1,2,3 |
| 16 | 1033 | 1,8-Cineole | 0,66 | 15,85 | 5,39 | 1,2,3 |
| 17 | 1040 | cis-Ocimene | 4,25 | 2,67 | 1,35 | 1,2,3 |
| 18 | 1050 | trans-Ocimene | 2,73 | 1,90 | 2,08 | 2,3 |
| 19 | 1062 | γ-Terpinene | 0,16 | 0,25 | - | 1,2,3 |
| 20 | 1068 | cis-Sabinene hydrate | - | - | 0,06 | 2,3 |
| 21 | 1064 | cis-Linalool oxide | - | - | 0,08 | 1,2,3 |
| 22 | 1088 | Terpinolene | - | 0,33 | 0,28 | 1,2,3 |
| 23 | 1097 | trans-Sabinene hydrate | - | 0,34 | - | 2,3 |
| 24 | 1098 | Linalool | 50,63 | 23,01 | 37,69 | 1,2,3 |
| 25 | | Propionic acid hexyl est. | - | 0,13 | 0,15 | 1,2,3 |
| 26 | 1110 | Octen-3-yl-acetate | 0,10 | - | 0,30 | 1,2,3 |
| 27 | 1124 | 3-Octanol acetate | - | - | 0,12 | 2,3 |
| 28 | 1129 | allo-Ocimene | 0,02 | - | - | 1,2,3 |
| 29 | 1143 | Camphor | 0,06 | 11,35 | 5,03 | 1,2,3 |
| 30 | 1150 | Hexyl isobutyrate | - | 0,17 | 0,17 | 1,2,3 |
| 31 | 1165 | Borneol | 0,34 | 1,29 | 1,71 | 1,2,3 |
| 32 | 1166 | Lavandulol | 1,52 | - | 0,14 | 1,2,3 |
| 33 | 1177 | Terpinen-4-ol | 7,84 | 6,67 | 0,08 | 1,2,3 |
| 34 | | Cryptonel | 1,20 | 0,28 | 0,22 | 2,3 |
| 35 | 1189 | α-Terpineol | 1,52 | 3,81 | 2,91 | 1,2,3 |
| 36 | 1191 | Hexyl butyrate | - | 0,69 | 0,82 | 1,2,3 |

Continued

| Nr | KI | Constituents | *Lavandula angustifolia* | *"Super"* | *"Special"* | Method of dentification |
|---|---|---|---|---|---|---|
| | | | Percentage yield | | | |
| 37 | 1211 | 1-Octanol acetate | - | 0,05 | 0,06 | 2,3 |
| 38 | 1228 | Nerol | 0,33 | 0,43 | 0,53 | 1,2,3 |
| 39 | 1239 | Cumin aldehyde | 0,28 | 0,09 | 0,05 | 1,2,3 |
| 40 | 1242 | Carvone | 0,09 | - | - | 1,2,3 |
| 41 | 1243 | Hexyl isovalerate | - | 0,08 | 0,10 | 1,2,3 |
| 42 | 1257 | Linalyl acetate | 15,72 | 20,35 | 29,14 | 1,2,3 |
| 43 | 1285 | Bornyl acetate | - | 0,08 | - | 1,2,3 |
| 44 | 1287 | Cumin alcohol | 0,13 | - | - | 1,2,3 |
| 45 | 1289 | Lavandulyl acetate | 2,73 | 0,37 | 2,20 | 1,2,3 |
| 46 | 1331 | Hexyl tiglate | - | - | 0,21 | 1,2,3 |
| 47 | 1365 | Neryl acetate | 0,50 | 0,66 | 0,83 | 1,2,3 |
| 48 | 1383 | Geranyl acetate | 0,93 | 1,34 | 1,56 | 1,2,3 |
| 49 | 1418 | β-Caryophyllene | 2,04 | 0,91 | 0,55 | 1,2,3 |
| 50 | 1420 | α-Santalene | 0,35 | 0,09 | - | 2,3 |
| 51 | 1436 | trans-α-Bergamotene | 0,11 | 0,03 | - | 2,3 |
| 52 | 1454 | α-Humulene | 0,06 | - | - | 1,2,3 |
| 53 | 1458 | β-Farnesene | 0,07 | 0,25 | 0,35 | 2,3 |
| 54 | 1480 | Germacrene-D | 0,24 | 0,30 | 0,23 | 2,3 |
| 55 | 1508 | α-Farnesene | - | - | 0,03 | 2,3 |
| 56 | 1581 | Caryophyllene oxide | 0,31 | 0,13 | 0,07 | 1,2,3 |
| 57 | 1640 | τ-Cadinol | 0,06 | 0,06 | 0,08 | 2,3 |
| 58 | 1653 | α-Cadinol | - | 0,03 | 0,01 | 2,3 |
| 59 | 1683 | α-Bisabolol | - | 1,63 | 1,09 | 1,2,3 |

[a] Elution order from the DB-5 column

[b] Kovats retention index on a DB-5 column

[c] 1 authentic sample (mainly from Roth and Aldrich, Sigma), 2 reference oils, 3 mass spectra

3) as final confirmation of the peak identification by GC/MS comparing their spectra with those in a commercial library Mass Spectra Database Newterp and NIST 98. The analysis was performed on a fused silica column DB-5, 30m x 0.25 mm i.d., film thickness 0.25 μm (J & W Scientific Inc., Rancho Cordova, California, USA) using a Gas Chromatograph 17A Ver. 3 interfaced with a Mass Spectrometer Shimadzu QP-5050A supported by the Class 5000 software. Injection temperature: $260^0$; interface heating: $300^0$; ion source heating: $200^0$; EI mode: 70eV; scan range: 41 – 450 amu; and scan time 0.50 s. Oven temperature programmes: a) $55^0$ – $120^0$C ($3^0$/min), 120-$200^0$C $4^0$/min, 200 - $220^0$ ($6^0$/min) and $220^0$ for 5 min and b) 60 - $240^0$ at $3^0$ C/min, carrier gas He, 54.8 kPa, split ratio 1:30.

**Table 2.** Significant compounds and their percentages in the essential oils from *Lavandula angustifolia* and of the hybrids "*Super*" and "*Special*" from Greece

| Constituents | L. *angustifolia* | "*Super*" | "*Special*" |
|---|---|---|---|
| | Percentage yield | | |
| 1,8 Cineol | 0,66 | 15,85 | 5,39 |
| Linalool | 50,63 | 23,01 | 37,69 |
| Camphor | 0,06 | 11,35 | 5,03 |
| Borneol | 0,34 | 1,29 | 1,71 |
| Linalyl acetate | 15,72 | 20,35 | 29,14 |
| Nerol | 0,33 | 0,43 | 0,53 |
| Neryl acetate | 0,5 | 0,66 | 0,83 |
| Geranyl acetate | 0,93 | 1,34 | 1,56 |
| Rhodinol fraction | 1,76 | 2,43 | 2,92 |

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