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Evolution of camphor and others components in the essential oils of two labiate species during the biological cycle

J. Kaloustian*, A.-M. Pauli and J. Pastor

Laboratoire de Chimie Analytique, Faculté de Pharmacie, Université de la Méditerranée, 27, boulevard Jean Moulin, 13385 Marseille Cedex, France

Abstract. Essential oils are used in perfumes, cooking and in pharmacy. A special study had been done on essential oils issued from two plants of Lavendin and Rosemary, during the annual biological cycle. The GC/MS technique was used and identification of separated components was achieved by retention times in comparison with respective standards and by mass spectra comparison. Quantitative determinations of camphor, cineol, linalool, linalyl acetate in Lavendin, and camphor, α-pinene, cineol in Rosemary, were done by GC/FID. *n*-Dodecane was used as internal standard. The aim of this study was to choose the optimum period of plant gathering, with respect to the level of camphor and other major components. An increasing of the camphor is observed in the leaves. Camphor, present in essential oils of Lavendin and Rosemary, has new pharmaceutical applications, mainly as stimulant, cardiac and respiratory analeptic.

Keywords. Camphor – essential oils – GC/MS – GC/FID – Lavendin – Rosemary.

Introduction

Very often, one hears, in media, the apology of the way of life in Provence (French paradox), and generally, in the Mediterranean basin. It is put forward the consumption of fresh vegetables and fruits… but also olive oil, and especially aromatics collected in the "Provençale garrigue". Certainly, it is known for a long time of the kindness of these aromatics and their essential oils in the domain of Health and Perfume. The essential oils are the smelling substances secreted by the specialised cells of some plant organs: flowers, leaves, branches, fruits…

The aromatherapy is a part of the phytotherapy, using some aromatic plants for disease treatments. The inhalation seems to be the most convenient way, as well as the oral or external way. The popular medicine uses it for antibacterial and antifongic activities. Oil can be absorbed by skin (bath, massage…).

In the case of Lavendin and Rosemary, the branches are deprived of oil. The oils are get from flowers and leaves, by steam distillation. The oils are some limpid, water insoluble, colourless or weakly yellow colourful liquids with specific odours.

We will present the study of two species of labiate (Lavendin, Rosemary) and their essential oils. Recent studies showed some uses in the unsuspected therapies until now.

The Lavender name (*Lavandula*) could have for origin the Latin verb "Lavarer" (to wash), what would confirm its reputation of antiseptic. It was especially applied in frictions and added to baths by the Romans [1-3].

The Lavender belongs to the Labiate family. Numerous species exist [4]. The most used are:

– the Lavender or French Lavender or vera Lavender (*Lavandula Angustifolia*),

– the spice Lavender (*Lavandula Latifolia*).

The Lavendin is a hybrid of these two Lavenders [5]. Several clones exist:

– the Lavendin Grosso, the most frequent,

– the Lavendin Super, nearest of the *Lavandula Angustifolia*,

– the Lavendin Abrial, which blooms shortly after the *Lavandula Angustifolia*.

The *Lavandula Angustifolia* provides oil used by the perfumers. The creation of new needs in soaps, laundries and detergents had increased the cultures of Lavendin.

Until now, the majority of researches had been carried on the utilization of the essential oil of the vera Lavender in the food and perfume industries. But little research had been made for its utilization in the domain of Health. Until now, only the oil of vera Lavender was known to have some therapeutic properties, contrary to the Lavendin that would not have. We will recall some properties [6-7]:

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- in the treatment of wounds by a healing action ;
- as antispasmodic, while facilitating the digestion ;
- as relaxing, sedative and even as analgesic ;
- as hypotensive.

It is without danger for babies and pregnant women. Some recent studies in paediatrics showed a better relaxation of the new-born, when one uses some drops in the baths [8].

In an intensive care unit, the emotional stress affects the convalescence. Massages can improve the state of relaxation, but the number of essential oils is high, and each one is supposed to be specific for its therapeutic qualities. Massages of members and the forehead had been achieved on post-cardiotomy patients. The relaxing effect is more important when one uses Lavendin essential oil, rather than the Lavender one [7]. It would seem that the presence of camphor, volatile product and quickly inhaled, can play a role of stimulant. Traditionally, and in an extensive international literature search for this study, the Lavendin was discredited as being of no use therapeutically. However, if the results of above-mentioned research are duplicated, it would appear that the Lavandin oils have good therapeutic actions.

The Rosemary name comes from “Ros” and “Marinus” which means “marine dew”. The Rosemary is known for a long time. The Greeks and the Romans burnt it as incense [9].

The first flowers appear at the end of the winter, and are renewed always until the beginning of the summer. A modestly blossoming is present also in autumn.

The leaves are used mainly in cooking. The Rosemary tea has good therapeutic properties: antiseptic, antispasmodic, diuretic; the essential oil is used in external way as antiseptic mouth wash [10], but also as antidiarrhoeic, antirheumatic, analgesic, healing [11]. The essential oil must not be used in the case of pregnancy, epilepsy. It can be allergic, after body sun exposure. In France, several pharmaceutical specialities (like Aromasol™, Humex Revulene™, …) are used in respiratory disease. New searches were run for the valuation of antimalarial [12], antifongic [13] and antiparasitic (*Pediculus humanus*) [14] activities.

The chromatographic curves of these oils, used in pharmaceutical applications, are known for a long time but some components, like camphor, might have a specific therapeutic action.

The goal of this work is the application of gas-liquid chromatography to the analysis of essential oils obtained from Lavendin and Rosemary by steam distillation, in our laboratory, from several samples harvested on the same plants of each labiate species, during the biological cycle: autumn, winter, spring and summer. It might be possible to choose the period and the analysed aerial part, for an optimum level of some determinated components.

Experimental

Sampling

The studied samples had been gathered on the same plants during their annual biological cycle:

- Lavendin, in Aix-en-Provence (Bouches-du-Rhône),
- Rosemary, in a village (Entrevennes) near Manosque (Alpes-de-Haute-Provence).

The gathering dates are characterised by six digits: day, day, month, month, year, year. Some hours after the picking, leaves branches and flowers are separated.

The study on the Lavendin had been run in the period of 04.01.1998 to 25.08.1998 days. During the first one (January to June) the plant had no flowers. Flowers were present in the second period (June to August). The study on the Rosemary had been run between the 22.11.1998 and 26.06.1999 days. The flowers had been always present, but sometimes in very few quantities, with no possible titration.

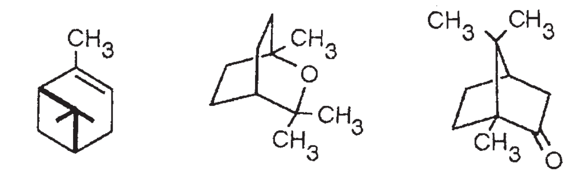
First, we determinate the essential oil on a part of the freshly picked plants, according to the French Pharmacopoeia. Another part is dried at 110 °C within fifteen hours, with the determination of the weight loss. During the heating, the essential oil is almost completely eliminated (about 99 %). All the results are presented according to the dry matter (%/DM).

Secondly, the oil’s components are characterised by GC-MS, in comparing retention times and MS curves with standards.

Finally, several oil’s components, among the most important, had been titrated (internal standardisation with *n*-dodecane) by GC-FID:

- Lavendin: camphor, cineol, linalool, linalyl acetate ;
- Rosemery: camphor, cineol, α-pinene.

The chemical formulas are reported in figure 1.



α-pinene cineol camphor

$(CH_3)_2C{=}CHCH_2CH_2C(CH_3)(OH)CH{=}CH_2$

linalool

$CH_3CO_2C(CH{=}CH_2)(CH_3)CH_2CH_2CH{=}C(CH_3)_2$

linalyl acetate

Figure 1. Chemical formulas of determinated components in essential oils.

Conditions

Two Gas-Liquid Chromatography techniques had been used.

– Qualitative analysis: GC/MS was carried out using total ion monitoring mode on a Varian 3400 apparatus, with a detector MS ion trap. The column HP5MS (copolymer diphenyl 5 % – dimethyl 95 % siloxan; 30 m length × 0.32 mm inside diameter, 1.0 µm film thickness) in solid support silica was used. The heating rate was 3 °C/min from 60 to 200 °C. Injector temperature was set at 250 °C. Helium was used as carrier gas with a flow rate of 1.0 mL/min. The standard solutions and the essential oils solutions were in hexane (1 µL/mL and 2 µL/mL).

  Unfortunately, the quantitative analysis could not be run on GC/MS apparatus.

– Quantitative analysis: GC/FID Hewlett Packard 5700 apparatus was used. The column (2 m length × 0.32 cm inside diameter, solid support glass, Chromosorb 80/100 Mesh, OV225) was held at 50 °C for 4 min and programmed at 8 °C.min to 200 °C. Injector and detector FID temperatures were 250 °C. The flow rates were 30 mL/min for hydrogen, 240 mL.min for air and 8.7 mL/min for nitrogen carrier gas. *n*-Dodecane was used as internal standard. Calibration curves were obtained by plotting the ratio peak area analysed component/peak area *n*-dodecane, *versus* the amounts of components. Internal standard was at 0.75 mg/mL. For the calibration curves of each component, 6 standard solutions from 0 to 1.5 mg/mL in hexane were used as data points. In each case (α-pinene, cineol, camphor, linalool, linalyl acetate) regression data slope was computed, a linear fit was satisfactory with a correlation coefficient *r* always upper 0.992.

  The standards are from Aldrich-Chemie Company.

Results

Lavendin

The determination of the essential oil of leaves and flowers, issued from the same plant of Lavendin, is presented in table I (results according to the dry matter, in ml/100 g DM).

Table I. Determination of essential oil in the samples issued from the same plant of Lavendin (in ml/100 g DM).

Samples	*Leaves*	*Flowers*
1 – 040198	0.80	Absent
2 – 010298	0.42	Absent
3 – 150298	0.77	Absent
4 – 080398	0.39	Absent
5 – 070698	0.54	7.99
6 – 020798	0.49	9.74
7 – 250898	ND	9.44

ND = Not determinated, very little quantity of leaves

For all the samples of branches issued from all lots, the oil determinated according to this procedure is always, either absent, or lower than 0.06 ml/100 g DM. The results of the leaves are included between 0.39 and 0.80 ml/100 g DM. The flowers present an upper level at 9.74 ml/100 g DM when they were completely opened. This level is at 7.99 when they are scarcely opened and 9.44 when they are faded without petals.

The identification of sixteen components among the most characteristic, was done by GC-MS, according to their retention time (expressed in seconds) and their mass spectrum. The figures 2A (purchased Lavendin oil) and 2B (Lavendin flowers oil of our sampling) present similar curves, with mainly, linalool and linalyl acetate upper levels. The Lavendin leaves oil of our sampling (Figure 2C) shows very little values of linalool and its acetate ester, but upper levels of camphor and cineol.

The oil component titration by GC-FID (internal standard *n*-dodecane) of four samples issued from our sampling is presented in table II. We add the results of *Lavandula dentata* leaves (260198), which has the particularity to blossom in winter. We present, in figure 3, an example of chromatographic curve (GC/FID) obtained on Lavendin flowers essential oil with the use of *n*-dodecane as internal standard.

The industrial samples are the bloom heads gathered mechanically when the flowers are fully opened. These bloom heads are formed mainly of flowers and branches. Few leaves are present.

Table II. Titration of the four components of Lavendin and *Lavendula dentata* oils (% w/w).

Samples	*Cineol*	*Linalool*	*Camphor*	*Linalyl Acetate*
Obtained From Industries				
A Lavendin Colourless Oil	4.11	37.0	2.67	38.9
B Lavendin Colourless Oil	4.68	38.2	4.02	37.2
C Lavendin Yellowish and aged Oil	3.06	29.6	6.54	34.8
D Lavendin Yellow and aged Oil	3.68	22.9	6.82	36.4
Obtained From our Sampling				
1 – 040198 leaves	9.03	–	22.0	–
2 – 010298 leaves	16.3	–	34.2	–
3 – 150298 leaves	20.5	–	35.3	–
4 – 080398 leaves	16.2	–	30.9	–
5 – 070698 leaves	19.0	1.60	29.9	–
5 – 070698 flowers	8.49	21.4	11.2	21.5
6 – 020798 flowers	8.61	28.9	9.22	29.1
7 – 250898 flowers	8.13	36.0	13.9	17.3
Lavandula Dentata Leaves 260198	1.12	5.81	27.8	–

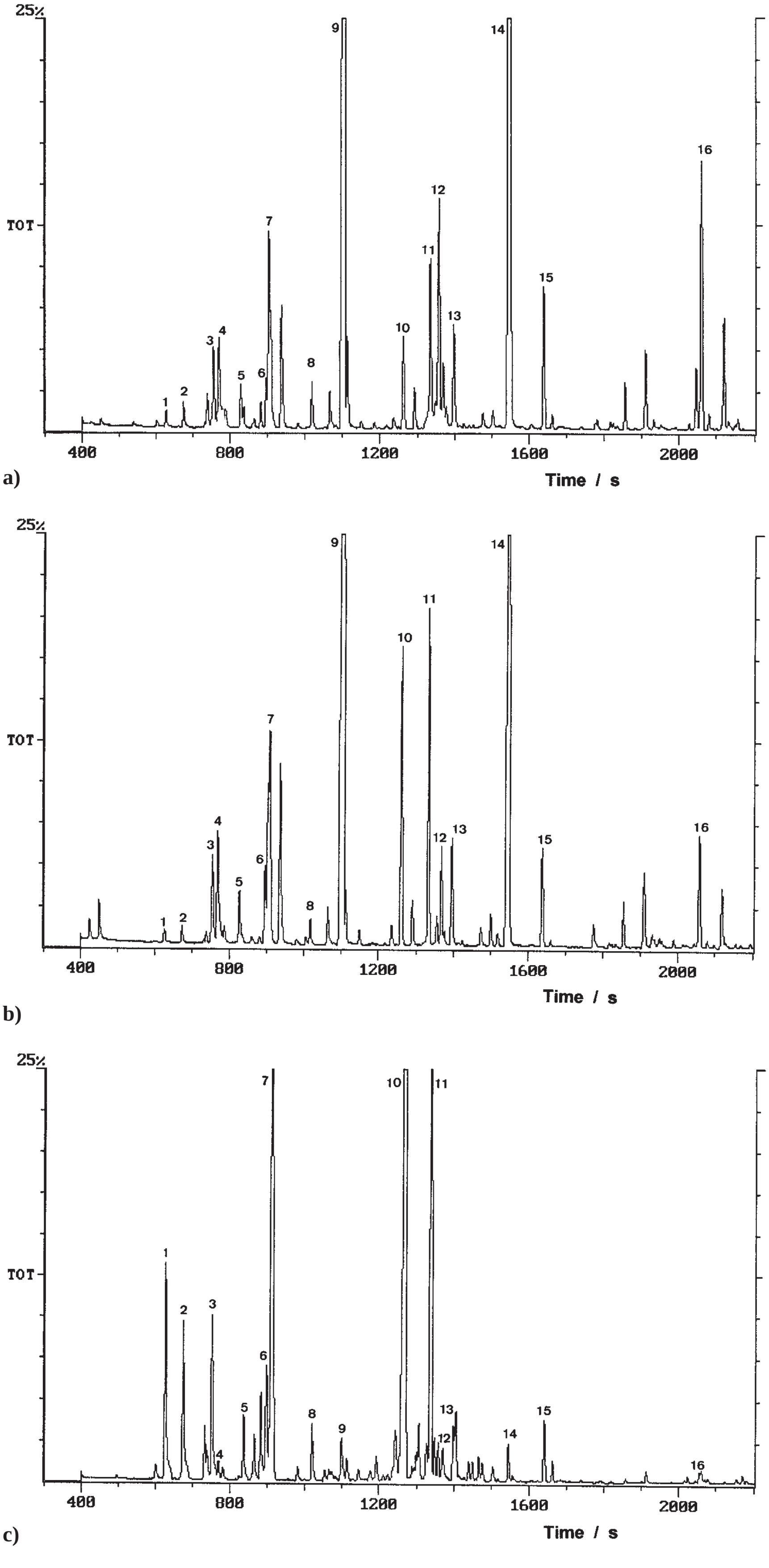


Figure 2. Qualitative analyses-GC curves of essential oils. A = Lavendin, obtained by buying in the shops; B = Flowers of Lavendin (our sampling); C = Leaves of Lavendin (our sampling); 1 = α-pinene, 2 = camphene, 3 = β-pinene, 4 = myrcene, 5 = cymene, 6 = limonene, 7 = cineol, 8 = linalool oxide, 9 = linalool, 10 = camphor, 11 = borneol, 12 = terpineol-4, 13 = α-terpineol, 14 = linalyl acetate, 15 = geranyl acetate, 16 = trans (or β)-caryophyllene.

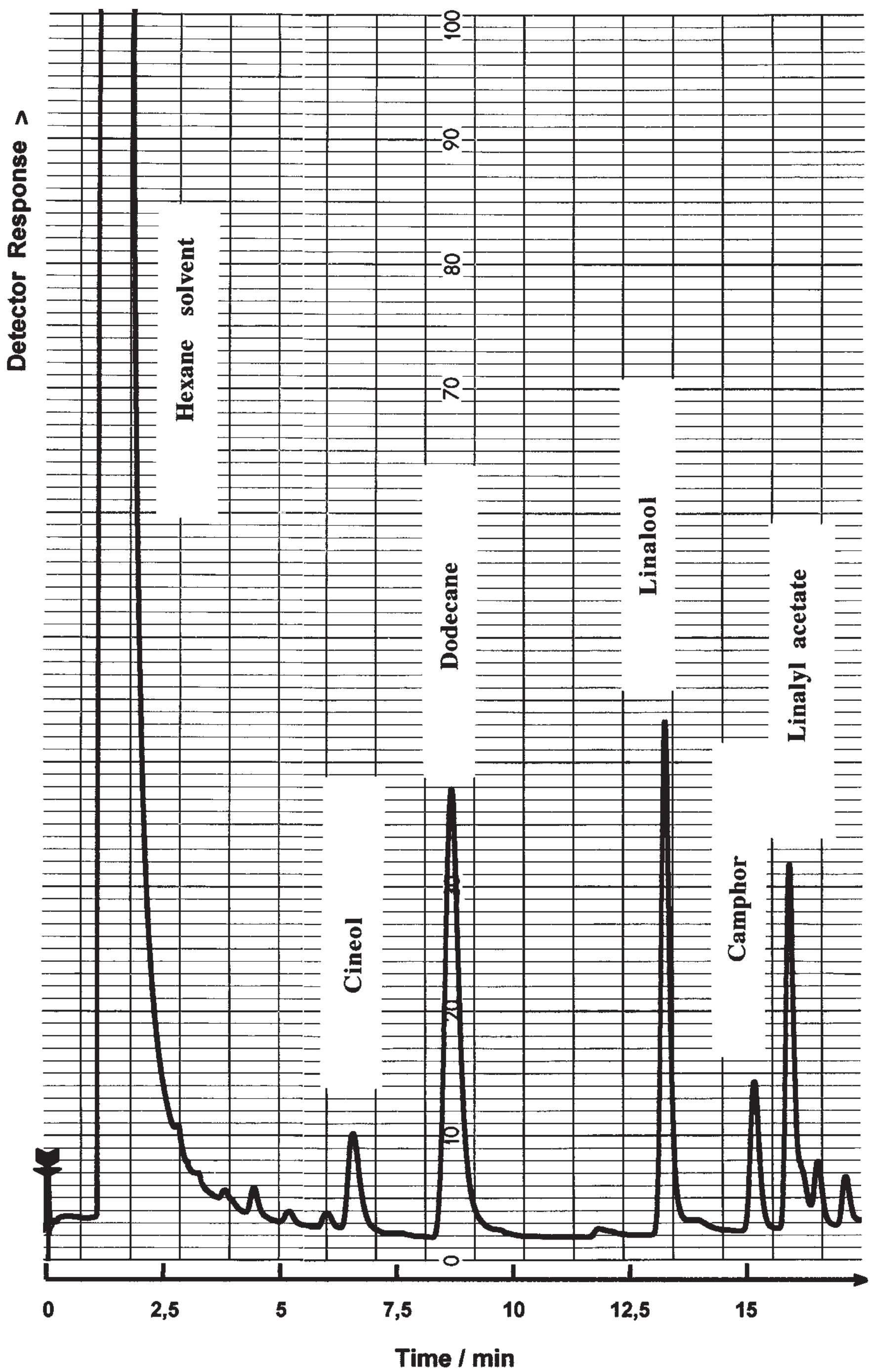


Figure 3. Quantitative analysis-GC curve of Lavendin flowers essential oil.

The comparison of the four oils issued from industries shows near values of the A and B Lavendin oils. The samples C and D are aged Lavendin oils. The linalool and linalyl acetate levels are lower. On contrary the camphor is higher.

In February, camphor is at upper level in the leaves oils obtained from our sampling. In July, the flowers oil presents 29 % of linalool and linalyl acetate. On contrary, cineol and camphor levels are at about 9 %. During the flowers ageing (not opened, to faded and without petals) the linalool level increases from 21.4 to 36.0 %. The increasing (28.9 to 36.0 %) of the linalool, corresponds to the decreasing of linalyl acetate: 29.1 to 17.3 % (ester hydrolysis).

Rosemary

10 tests of essential oil determination were made, by steam distillation, on a sample containing only leaves and flowers, without branches, and coming from another plant of

Rosemary. The computed average was 1.95 ml/100 g Dry Matter, with a standard deviation of 0.107, and a correlation coefficient of 5.50 %.

The results of the 7 samples gathered on the same plant are presented in table III.

We observe for the leaves of Rosemary an increasing of the essential oil from 2.65 (November) to 3.59 ml/100 g DM (June). The two maximums, observed for samples 3 and 7, can be explained by a lower pluviometry in December and in June (Fig. 4). It is not surprising to establish for the lots 3 and 7 (January 6 and June 26) higher quantities of essential oils (dryness in the preceding days).

Table III. Determination of essential oils in the samples issued from the same plant of Rosemary (in ml/100 g DM).

Samples	*Leaves*	*Flowers*	*Whole branches*
1 – 221198	2.65	2.47	1.73
2 – 101298	2.84	1.86	2.12
3 – 090199	3.15	1.85	1.86
4 – 060299	3.05	ND	1.82
5 – 060399	2.84	ND	2.15
6 – 040499	2.97	ND	2.21
7 – 260699	3.59	ND	3.23

ND = Not determinated, very little quantity of flowers

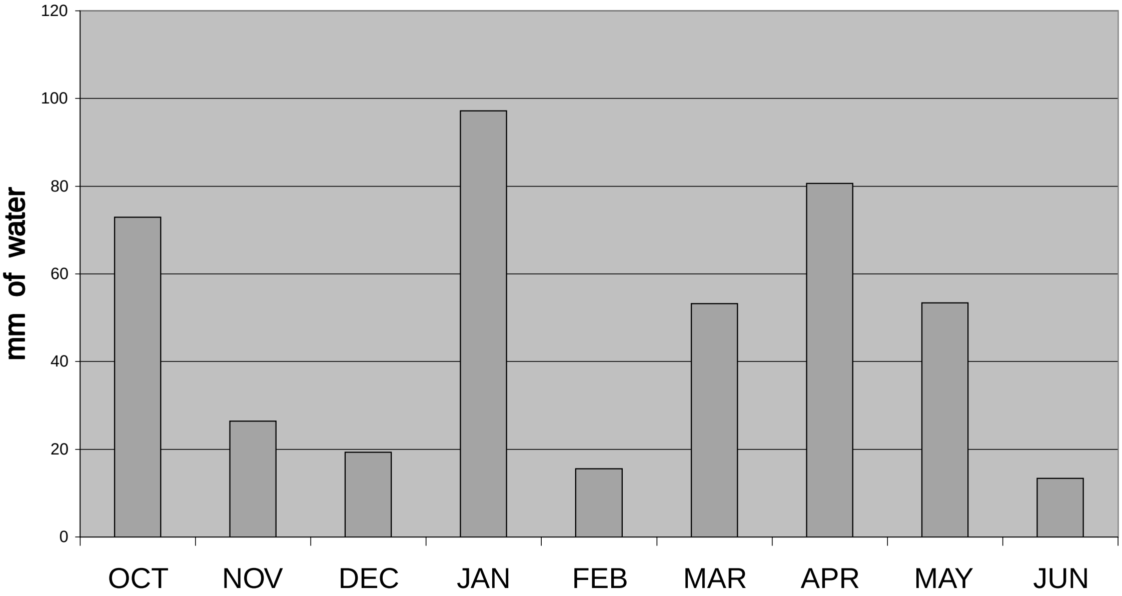


Figure 4. Pluviometry in Manosque (1998-1999).

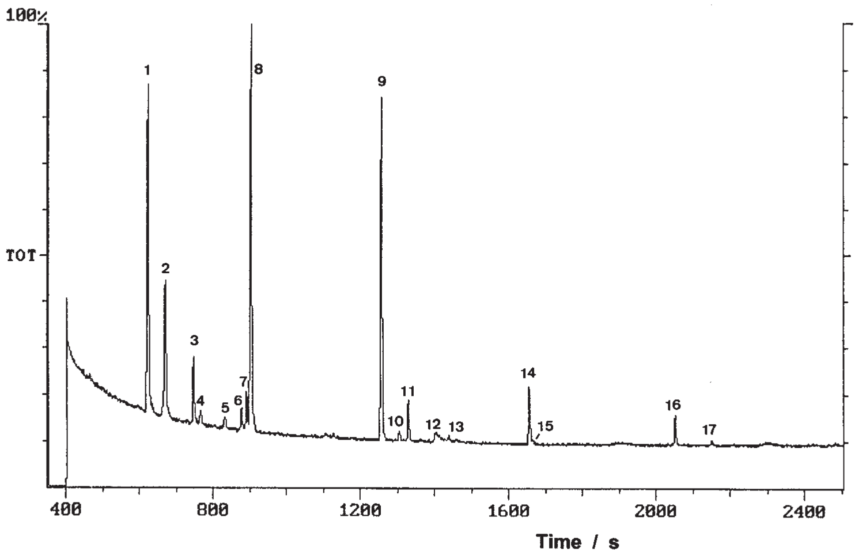


Figure 5. Qualitative analysis- GC curves of Rosmarinus officinalis oil. 1 = α-pinene, 2 = camphene, 3 = β-pinene, 4 = myrcene, 5 = Δ-3 carene, 6 = cymene, 7 = limonene, 8 = cineol, 9 = camphor, 10 = isoborneol, 11 = borneol, 12 = α-terpineol, 13 = verbenone, 14 = bornyl acetate, 15 = isobornyl acetate, 16 = trans (or β)-caryophyllene, 17 = α-humulene

Table IV. Repetability of the titration of three components in an homogeneous mixture of leaves and flowers (% w/w).

	α-Pinene	*Cineol*	*Camphor*
Average (% w/w)	4.77	15.2	28.8
SD	0.106	0.198	0.312
CV (%)	2.23	1.30	1.08

The identification of seventeen components in the Rosmarinus officinalis essential oil was done by GC/MS, according to their retention time (expressed in seconds) and their mass spectrum (Fig. 5). The same chromatographic curves were observed for the oils issued from our sampling (leaves, flowers, whole branches) and the *Rosmarinus officinalis* oil obtained from industries.

In order to verify the repetability of the essential oil titration, we had run 10 tests on the same lot: homogeneous mixture of leaves and flowers. The average (in %), the standard deviation (SD) and the correlation coefficient (CV in %) were computed, according to three important components of the oil: α-pinene, cineol, camphor (Tab. IV).

The correlation coefficient is included in the interval 1.1 to 2.2 %. All the titration results, according to the essential oils issued from our sampling and to a sample of Rosmarinus officinalis obtained from industries, are in table V.

The flowers were not always in sufficient quantities for the determination of the oil. We add the results of the new leaves of the lot 7: α-pinene 11.2 % ; cineol 19.8 % and camphor 34.9 %. These values are near the values of the leaves coming from the precedent year and mentioned in table V.

In our Rosemary's sampling of leaves and flowers, the levels of the three tested components are near. In contrary, we observed in Lavendin, significative variations betwen leaves and flowers. In the *Rosmarinus officinalis* oil each of these components: α-pinene, cineol and camphor, corresponds to about a quarter (in % w/w) of the essential oil, with a similarity to Spanish Rosemary [15]. In our studied samples, α-pinene is lower than 10 %, cineol between 13 and 21 %; camphor has a level always upper than 30 %, but lower than 45 %.

Table V. Titration of the three components of the Rosemary and *Rosmarinus officinalis* oils (% w/w).

Samples	*α-Pinene (%)*	*Cineol (%)*	*Camphor (%)*
Rosmarinus officinalis	25.5	29.1	23.1
Obtained from our Sampling			
1 – 221198 leaves	4.62	13.4	31.1
1 – 221198 flowers	9.95	15.4	34.8
2 – 101298 leaves	4.12	13.4	31.9
3 – 090199 leaves	8.44	21.3	41.4
3 – 090199 flowers	9.64	16.6	39.9
4 – 060299 leaves	8.29	18.7	44.5
5 – 060399 leaves	8.53	19.3	41.0
6 – 040499 leaves	7.79	18.8	41.7
7 – 260699 leaves	7.86	19.2	37.3

Table VI. Titration of the three components of the Rosemary oils (g/100g dry matter).

Samples	*α-Pinene (g/100 g DM)*	*Cineol (g/100 g DM)*	*Camphor (g/100 g DM)*
1 – 221198 leaves	0.110	0.320	0.743
2 – 101298 leaves	0.105	0.320	0.816
3 – 090199 leaves	0.239	0.604	1.17
4 – 060299 leaves	0.228	0.514	1.22
5 – 060399 leaves	0.218	0.494	1.05
6 – 040499 leaves	0.208	0.503	1.11
7 – 260699 leaves	0.254	0.620	1.20

In the leaves, the upper level of each component in the oil is observed, for the cineol in January, camphor in February and α-pinene in March. The table V shows the results of the three components in the oils, in % weight/weight. But the oil value is not constant in the aerial parts (leaves, flowers, whole branches) according each lot. If we consider a middle density of 0.90 [5] and with the knowledge of the humidity present in the samples, we can compute approximative results of α-pinene, cineol and camphor, according to the dry matter (in g/100 g DM), (see table VI). If we take into account the concentration of these components (in g/100 g DM), a maximum is observed in June.

The whole branches (with leaves, flowers and boughs) present some random values due to the presence of boughs with various diameters, and so various weights in the samples, carrying differences in the oil determination. So, we will not use the results coming from the whole branches.

Discussion

In our sampling of Lavendin, we observe an upper level of camphor in flowers (13.9 %) when they are faded. In leaves, the content of camphor is upper in February (34-35 %). The essential oils of the Lavendins and vera Lavender have near levels of linalool and linalyl acetate; in vera Lavender, the levels of cineol and camphor are lower than 1.5 %, on contrary, upper in the Lavendins [4]. Our studied sample should be the hybrid Lavendin Abrial, because the observed values, in the case of the opened flowers and gathered in July, are corresponding to the composition cited by the French norms [5]: cineol 6-11 %, linalool 26-38 %, camphor 7-11 %, linalyl acetate 20-29 %.

If the steam distillation is done on all branches with flowers and mainly leaves placed at the base of the plant, one will observe more camphor and cineol (coming from the leaves) in the essential oil. This oil might be more efficacious in the case of therapeutic massages and/or in inhalation.

Literature mentions an increasing of essential oil issued from the leaves, in comparison with the flowers of Rosemary [16]. The blooming of the first flowers takes place at the end of winter. This phenomenum renews at once until during spring. In autumn, the blowing is modester [10]. Moreover, the oil issued from the leaves is in more quantity before the blooming [17]. That explains our results with a maximum of oil for the lots 3 and 7. It seems that the studied plant of Rosemary, can draw near the Hungarian variety which resists to freezing, with an oil composition: α-pinene 4-14 %, cineol 21-46 % and camphor 13-31 % [18]. The studied plant grows at an altitude of 1000 metres. In these conditions and in High Provence, the *Rosmarinus officinalis* has some difficulties to persist. The geographic environment, the soil, the climate, can have an affect on the chemical oil composition. In our case the gathering of the leaves in January and June will give an upper level in camphor.

Camphor is highly volatile. Any essential oil, which was inhaled would greatly affect the patient's response, as inhaled essential oil reach the brain almost instantly. Camphor is also a stimulant and, in the essential oil, it is also a cardiac and respiratory analeptic [7].

The use of essential oils coming from Lavendin and Rosemary, and with much more camphor, can be used in new pharmacological ways. This is the goal and the interest of this work in the potential use of new formulations, issued from plants, in the treatment of some diseases. The gas-liquid chromatography can characterize and value the components of these essential oils.

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References

1. Bonnier, G. *The big flora in colour*; Paris: Belin, 1990, volume 4.
2. Coste, H. *The descriptive and illustrated flora of France, Corsica and the adjacent regions*; Paris: Blanchard, 1990, volume 3.
3. Bayer, E. *Guide of the mediterranean flora*; Paris: Delachaux and Niestlé, 1990.
4. French Pharmacopoeia (10th edition) *Essential oil of French Lavender*, January 1989; *Essential oil of spike Lavender*, January 1989; *Essential oil of Lavendin Grosso*, January 1989.
5. Afnor (Association française de normalisation) *Compendium of French norms of essential oils*, *Essential oil of Lavendin Abrial*, NFT 75-303, October 1992 ; *Essential oil of Lavendin Grosso*, NFT 75-304, October 1992 ; *Essential oil of Lavendin Super*, NFT 75-305, July 1992.
6. Buckle, J. *Nursing Times* **1992**, *88*, 54-55.
7. Buckle, J. *Nursing Times* **1993**, *89*, 32-35.
8. Cornwell, S.; Dale, A. *Mod. Midwife* **1995**, *5*, 31-33.
9. Thomson, W.A.R. *The medicinal plants*; Paris: Berger-Levrault, 1981.
10. Cretti, L. *The aromatic and medicinal plants*; Paris: Atlas, 1981.
11. Pedrini, F.; Lucheroni, M.T. *The big book of the essential oils*; Paris: De Vecchi S.A., 1996.
12. Milhau, G.; Valentin, A.; Benoit, F.; Mallie, M.; Bastide, J.M.; Pelissier, Y.; Bessiere, J.M. *J. Essential Oil Research* **1997**, *9*, 329-333.
13. Perucci, S.; Mancianti, F.; Cioni, P.L.; Flamini, G.; Morelli, I.; Macchiono, G. *Planta Medica* **1994**, *60*, 184-187.
14. Mumcuoglu, K.Y.; Galun, R.; Bach, U.; Miller, J.; Magdassi, S. *Entomologia Experimentalis Applicata* **1996**, *78*, 309-312.
15. Analytical Method Committee *Analyst* **1997**, *122*, 1167-1174.
16. Boullard, B. *The nature of flavours and perfumes*; Paris: Estem, 1995.
17. Salle, J.-L. *The essential oils*; Paris: Frison-Roche, 1991.
18. Domokos, J.; Hethelyi; Palinkas, J.; Szirmai, S.; Tulok, M.H. *J. Essential Oil Research* **1997**, *9*, 41-45.