P34: NMR MARKERS FOR QUANTITATIVE ANALYSIS OF THE ROMANIAN LAVANDULA VARIETIES

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Abstract -Romania is among the top ten European countries in terms of plant production for pharmacological use. In our country, 29 varieties belonging to 17 species of medicinal and aromatic plants were approved (*Lavandula angustifolia* (Corbeanca /92)). *Lavanda angustifolia* is the most popular variety due to frost resistance.

In this paper, we established markers for quantitative analysis of the major components of Romanian *lavandula* varieties, based on ^{1}H-NMR spectroscopy. The spectral data combined with chemometric analysis served as validation tool for *lavandula* varieties.

Keywords: NMR, *Lavandula* varieties, quantitative analysis

1. INTRODUCTION

Essential oils are obtained from different parts of the plants: flowers, branches, leaves, wood, roots, or resins secreted by certain trees. Some plants have a high load in volatile oils, others are less concentrated, and thousands of kilograms of plant are sometimes needed to get one litre of essential oil.

Depending on this concentration, plant rarity or cropping/harvesting difficulties and plant properties, some 100% pure and natural essential oils are rare and sometimes very expensive [1].

Plants belonging to the same family, or the same species or even parts of the same plant generates essential oils with different properties. For example, spicy lavender essential oil has different properties by comparison with true lavender essential oil [2].

In Romania they are cultivated several species of *Lavandula*: French edible lavender, English common lavender, "Munstead" lavander, "Corbeanca" lavender, "Emilia" lavander and Lavandula X Intermedia Grosso (lavandin), hybrid obtained by crossing the *Lavanda angustifolia* (English lavender) with *Lavandula latifolia* (Portuguese lavender).

In the *Lavandula* species, more than 300 components have been identified over time. The main components of *lavandula* essential oil are linalool and linalyl acetate [3], as shown in table 1.

Table 1. Linalyl acetate and linalool content within the *lavandula* varieties studied

Lavandula variety	Linalool	Linalyl acetate
L. angustifolia	25-38%	25-45%
L. augustifolia x latifolia	24-37%	20-47%
L. latifolia	26-43%	1.5%

Lavandula latifolia also contains camphor and 1,8-cineol in appreciable amounts (5-14%, and 25-36% respectively), components that only appear in limited concentrations in fine lavender oil (<0,5% and 0 - 1,0% respectively). Lavandulol and lavandulyl acetate, compounds with irregular isoprenoid structure, are considered as "key" components for lavender and lavender oils because they are found only in trace amounts in *lavandula latifolia* oil [4].

2. EXPERIMENTAL

Markers for four major components of Romanian *lavandula* varieties have been established based on ^{1}H-NMR spectroscopy. The analysed essential oil (20 samples) were obtained in laboratory (from lavender plant cultivated in different regions of Romania) and respectively purchased from the Romanian market.

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Fig. 1 presents the regions from which the analyzed lavender species were harvested.

Figure 1. The distribution of the analyzed samples on the territory of Romania

Oil extraction: The oils were extracted by hydro-distillation 50 g of Lavender (stems and flowers) were placed in a 1000 ml round bottom flask with 500 ml water and the oils isolated over 3 h period using a Clevenger apparatus. The volatile oils were dried over anhydrous sodium sulphate and stored in sealed vials at 4° C until analysis.

NMR analysis: ^{1}H-NMR spectra were recorded on a Bruker Ascend 400 MHz spectrometer, operating at 9.4 Tesla corresponding to the resonance frequency of 400.13 MHz for the ^{1}H nucleus.

Quantitative ^{1}H-NMR measurement were performed using caffeine as internal standard, because its ^{1}H-NMR signals (methoxy groups and aromatic proton) are clearly distinguishable from those of the essential oil components. [5]

Samples were analyzed in 5 mm NMR tubes (Wilmad 507). Essential oil or references compounds were dissolved in stock solution of deuterated chloroform with caffeine (2:8 v/v). Recording of NMR spectra was performed using IconNMR software.

The average acquisition time of the ^{1}H-NMR spectra was approximately 2 minutes. The NMR spectra was recorded directly on the oil without any sample preparation.

3. RESULTS AND DISCUSSION

Identification of the major compounds within lavender essential oil, was made using reference compounds (linalool, linalyl acetat, camphor and eucalyptol) from Sigma Aldrich. To identify the corresponding signals to each major compound, a small amount of references compounds was added

in lavender essential oil. Fig. 2 presents ^{1}H-NMR spectrum of essential oil of lavender (1), linalool (2) and lavender essential oil with linalool (3). By comparison of the 3 NMR spectra, the characteristic markers for linalool have been selected (easily quantifiable and non-overlapping signals).
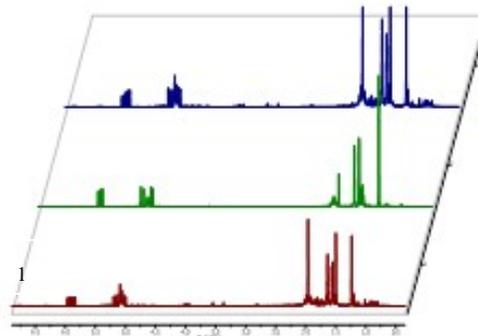

Figure 2. ^{1}H-NMR spectrum of essential oil of lavender (1), linalool (2) and lavender essential oil+linalool (3)

In a similar way were established characteristic signals for linalyl acetate, eucalyptol and camphor. Table 3 presents ^{1}H-NMR spectrum of essential oil of lavender and the major compounds identified.
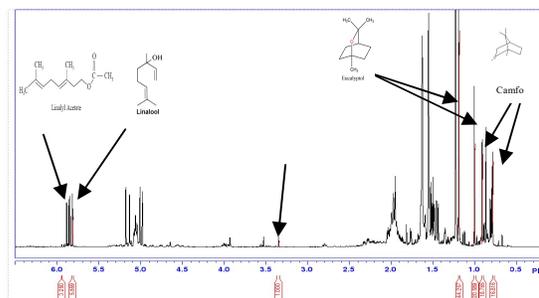

Figure 3. ^{1}H-NMR spectrum of essential oil of lavender

In Fig.4 the general profile of the 3 different varieties of Romanian *lavandula* is presented. As it can be seen there are some differences mainly in the signal ratio rather than the composition.
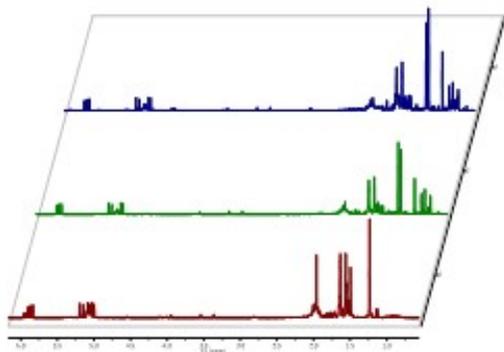
Figure 4. ^{1}H-NMR spectrum of *Lavandula Angustifolia (1), Latifolia (2)and AngustifoliXLatifolia (3)*

Based on spectral data obtained from ^{1}H-NMR spectrum we made chemometric analysis. Samples show a tendency to group according with the lavender variety.
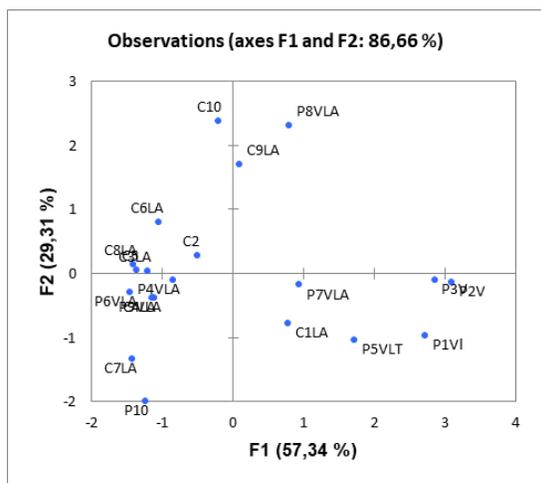

Figure 5. Principal component scores PC1/PC2 for essential oil of *Lavandaula* varieties studied

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