

## Research on volatile oil for lavender species

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**Abstract** Lavandula, Angustifolia, Mill, contain a volatile oil with a controlled chemical composition and which meets certain quality requirements. Objective of the paper is the chemical and biological characterization of some species, subspecies and varieties of Lavandula culture in Romania: Lavandula Angustifolia ssp. Angustifolia (LAA), Lavandula Angustifolia c.v. Hidicote Blue (LHB) and Lavandula Angustifolia Mill (LAM), etc.

The project theme is part of the issue that concern the European community and the national one, more precisely: The substantiation of the scientific knowledge regarding the lack of competitiveness of our vegetal material.

It is necessary to change a strategy on the culture line, by creating one Romanian variety or procurement of quality planting material, especially for Lavandula species.

### Key words

*Lavandula, Angustifolia, Mill., Hidicote*

Starting from the fact that the European Pharmacopoeia allows medical use exclusively for Lavandula Angustifolia Mill flowers (5), which contain a volatile oil with controlled chemical composition and meet specific quality requirements, the main purpose of my research consisted in chemical and biological description of some Lavandula species / subspecies / cultivars in Romania (1) they were as follows:

- Lavandula Angustifolia Mill. (LAM)
- Lavandula Angustifolia ssp. Angustifolia (LAA)
- Lavandula Angustifolia c.v. Hidicote Blue (LHB)

### Materials and Methods

Therefore, the research objectives consisted of:

- determination of morpho-anatomical features of Lavandula species / subspecies cultivated in Romania
- evaluation of volatile oil content extracted by hydrodistillation and determination of their chemical profile by gas chromatography analysis combined with mass spectrometry (GC-MS)
- monitoring the composition variability of volatile oil depending on climatic factors (year of culture, temperature and average depth of rainfall, solarization), location of origin, age of plant, the moment in the vegetation period when plant material samples are collected, type of the analyzed drug (flowers / inflorescences) – see image 1
- evaluation of pharmaceutical and aromatherapeutic quality of the separated volatile oils



Image 1 - Photos of Lavandula crop in Bălteni, Dâmbovița

The phytochemical analysis was based on thin-layer chromatography, gas chromatography combined with mass spectrometry, spectrophotometric determinations and high performance liquid chromatography (6).

The investigation of biological activity pursued two aspects:

- antioxidant activity
- antimicrobial and antifungal activity

In 2017, four volatile oils from *Lavandula* flowers from Burila-Mare, Mehedinți were chemically analyzed. The harvest of July 2017 analyzed the following leafy volatile oils:

- A - *Lavandula angustifolia* sep *Angustifolia*
- *Lavandula Angustifolia* c.v. Hidicote Blue
- *Lavandula Angustifolia* c.v. Munstend

B - three commercial lavender oils: Hofigal, Dacia plant and Faress

- the lavender reference from Bălteni-Dânbovița

Table 1

Characteristics of the gas chromatographic profile required by Ph. Eur. 6<sup>th</sup>

Chemical elements	Req.	Determination in %			
		LAA	LHB	LAM	LAP
linemen	under 1%	0.60	0.35	0.40	0.35
cineol	under 2,5%	-	-	-	-
camphor	under 1,2%	-	-	-	-
linalool	20,0-45,0%	24.22	28.22	25.11	25.10
linole acetate	25,0-46,0	13.31	0.09	13.28	11.13
4 terpineol	0,1-6,0%	9.68	8.12	-	4.94
lavandulyl acetate	above 0,2%	5.05	5.07	3.43	5.93
$\alpha$ -terpineol	sub 2,0%	4.23	3.07	5.12	3.51

Legend: *Lavandula angustifolia* sep *Angustifolia* - LAA ; *Lavandula Angustifolia* c.v. Hidicote Blue - LHB ;

*Lavandula Angustifolia* c.v. Munstend - LAM ; Commercial lavender oils: Hofigal, Dacia plant and Faress - LAP

The study continued with the qualitative and quantitative chemical analysis of *Lavandula* flowers, which took 2 years, when plant material collected from two different locations in Romania was analyzed: Balteni, county of Dambovița, Burila Mare, county of Mehedinți, monitoring the chemical composition of the volatile fraction and polyphenolic components (flavonoids, polyphenolcarboxylic acids and total polyphenols).

In the first phase, we explored the chemical composition of volatile oils (see in Table 1) separated from *Lavandula Angustifolia* Mill flowers harvested from Balteni in two consecutive years: 2015 and 2017. Although the soil, cultivation technology, even the

plants used to collect samples were the same in the two years, the chemical composition of the volatile oil separated in 2017 compared to the one in 2015 shows significant differences, mainly in terms of minor component subgroups: monoterpene hydrocarbons, sesquiterpene hydrocarbons and oxides.

In this situation, the only factors that could influence volatile oil's production and chemical composition were climatic factor. Monitoring the average monthly temperature variation in soil and air, rainfall and sunshine hours, we find that these could be determining factor for synthesis and different accumulation of terpenoids in plants.



Image 2 - Lavandula Angustifolia flowers



Image 3 - Bags of lavender and flowers

Perhaps the most important factor is the number and distribution of sunlight hours, 1,210 hours in 2015 compared to 1,261 in 2017, with peak intensity in the months of flower spikes development, as well as rainfall level, higher in May / June / July 2015 , as compared to the same period in 2017.

Since only monoterpene esters (linalyl acetate type) and monoterpenes (linalool type) are important for the pharmacological activity (2), we must admit that the mentioned climatic variations led in 2017 to a chemical composition of floral volatile oil deficient as compared to 2015.

Then our study aimed at highlighting the differences in chemical composition of the volatile fraction separated from the same plant material, fresh compared to dry material.

Although Eur.Ph. defines lavender volatile oil as substance extracted by hydrodistillation from dry flowers, there are producers in our country who process the fresh plant material, installing their mobile distillation plants in the summer on cultivated fields to save on transport and drying installations.

Seeking to establish the magnitude of composition differences in this case, we collected volatile oil from fresh or dried lavender flowers and tested it gas-chromatographically. The difference is obvious. Even if, at first glance, we could appreciate that the oil from fresh flowers is better due to its more complex composition, in reality the major components are diluted, the scent changes and the pharmacological activity is also attenuated.



Image 4 - Bags of Lavadula Angustifolia

Seeking to establish the extent of compositional differences reflected in the therapeutic effect, we laid down the mnemotechnic profiles for the two oils, fresh and dry, and compared them with the standard,

deducing then the range of pharmacological activity they are likely to develop.

The results are shown in table 2 and table 3.

Table 2

**Probable pharmacological activity of the two volatile oils and their intensity**

SOMATIC EFFECTS	Lavandula Angustifolia sspangustifolia		
	Standard	Fresh	Dry
Antibacterial, antiviral, antimycotic	++	+	++
Antipyretic	++	+	++
Strong immune stimulator	+++	+	++
Dermal regenerator, skin repair	+++	++	++
Anti-inflammatory	++	+	++
Blood circulation stimulant	++	+	++
Blood pressure regulator	+	+	++
Painkiller	++	+	++
Antispasmodic	++	+	++
Sleep inductor	++	+	++

Legend: very good activity (+++), good (++), weak (+), missing (-), dermal-irritant (\*)

Table 3

**Psychic Effects**

PSYCHIC EFFECTS	Lavandula Angustifolia sspangustifolia
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	Standard	Fresh	Dry
Balancing	+++	+	++
Sedative	+++	+	++
Anxiolytic and antidepressant	+++	-	+

Legend: very good activity (+++), good (++), weak (+), missing (-)

Table 4

#### Components identified by HPLC in polyphenolic fraction separated from flowers

Variants	Yg/ml extract								
	Chlorohydric acid	Cofeic acid	Ferulic acid	Rosmarinic acid	Cumaric acid	Catherina	Rutozida	Luteolina	Apigeonol
LAA	0.0001	0.0429	0.0637	0.0326	0.0002	0.0187	0.0940	0.0007	0.0007
LHB	0.0002	0.0312	0.0631	0.0223	0.0003	0.0169	0.0830	0.0007	0.0004
LAM	0.0001	0.0055	0.0581	0.0315	0.0002	0.0120	0.0834	0.0005	0.0006
LAP	0.0001	0.0355	0.0686	0.0172	0.0002	0.0196	0.0722	0.0011	0.0007
LHV	0.0001	0.0317	0.0634	0.0495	0.0004	0.0199	0.0538	0.0009	0.0004
LAV2	0.0003	0.0609	0.1008	0.0927	0.0004	0.0303	0.0906	0.0020	0.0005

Legend: Lavandula angustifolia sep Angustifolia-LAA, Lavandula Angustifolia c.v. Hidicote Blue-LHB, Lavandula Angustifolia c.v. Munstend-LAM, Commercial lavender oils: Hofigal, Dacia plant and Faress-LAP, LHV, LAV2

Color legend: LAA, LHB, LAM, LAP, LHV, LAV2

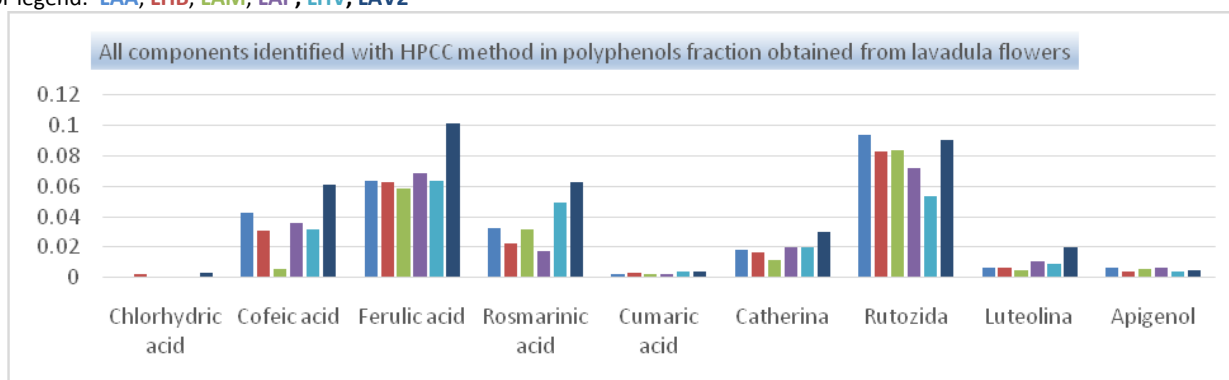


Figure 1. Graphical representation of the components identified by HPLC

From a quantitative point of view, the main components have been found to be Ferulic acid, Rosmarinic acid and Rutozida; the samples from Balteni-Dâmbovița are the richest in all analyzed components.

Going to study the biological activity of some separate fractions of Lavandula flowers, following investigations were performed:

- the antioxidant activity of the polyphenolic fraction, by the DPPH test and the determination of the quartz ionization capacity for the ferrous ion (4)
- the antimicrobial and antifungal effect of volatile oils (6)

Antioxidant activity was investigated for dry extracts, which is why we determined the total polyphenol content in mg gal. acid / 1g extract.

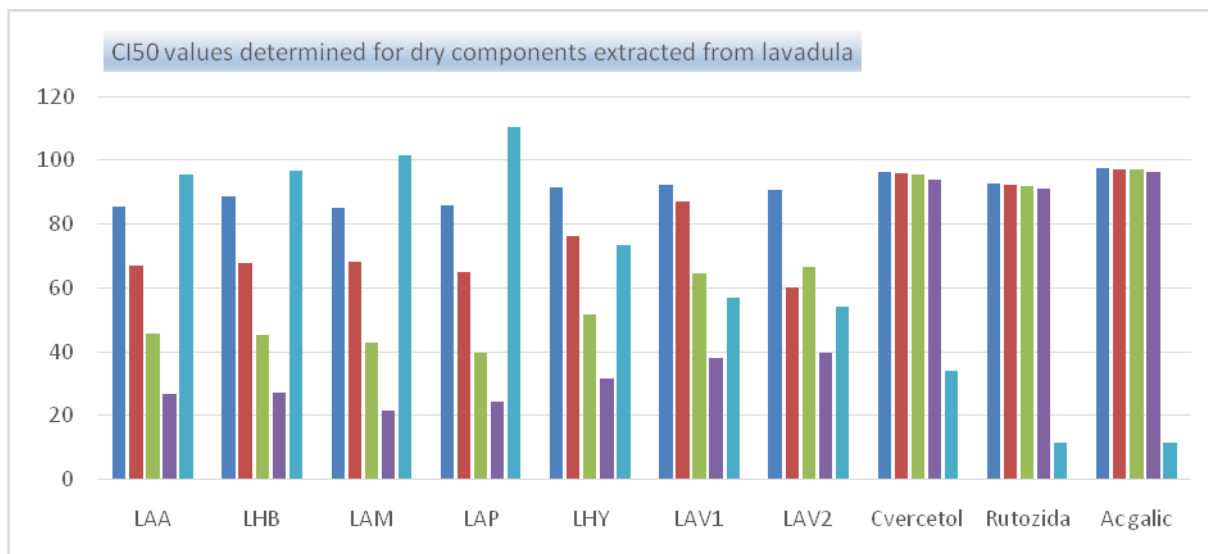


Figure 2. Graphical representation of the polyphenolic fraction by the DPPH test

CI50 determined for lavender dry extracts and cverceto, rutosida and galic acid were represented in Figure 2 above.

The strongest scavenging action is the lavender LAV1 and LAV2 samples from Dâmbovița, followed by LHY from Burila-Mare, Mehedinți. As in the previous analyses, by calculating CI50, the best chelating activity is LAV1 and LAV2, and the weakest LAP.

The antimicrobial and antifungal effect of volatile oils was determined on granulous bacteria and *Candida* genes, when MIC and CMM values were determined and synergistic effect was observed for the antibiotic or antifungal leaved oil associations (3).

If the antimicrobial effect of the lava oils tested proved to be of low to medium intensity, it is noted that the addition of volatile oil to the antibiotic induces a slight increase in bacterial activity. In all cases LHB is the one that has the best synergistic effect.

## Results

The pharmacology activity developed by standard volatile oil ranges from good to very well both somatically and mentally, their intensity is significantly reduced from weak to nonexistent for the sample of volatile oil separated from fresh flowers; besides, as can be seen, it is dermally irritating in topical application. Compared to this, the oil from the same flowers, but processed in dry state, recovers much of its pharmacological properties. Resuming studies in 2016, we sought to monitor the influence of certain factors on the quality of volatile oils.

The factors are as follows:

- Harvest time (July, August, October) – Bălteni (Dâmbovița county, România)

- The year of vegetation when the plant was found (second year, respectively third year)
- Burila-Mare county of Mehedinți where the plant was found (second year)
- Part of the analyzed plant (flower or floral stem) – Bălteni (Dâmbovița county, România)
- Climatic conditions – Burila-Mare (Mehedinți county, Romania)

## General conclusions

Paper's general conclusions can be structured as follows:

Macroscopically, all samples had in dry state the features required by the 6th European Pharmacopoeia. The histo-anatomical structure of the vegetative organs shows minor differences between the two subspecies (*L. Angustifolia* ssp. *Angustifolia* / *L. Angustifolia* Mill), rather quantitative differences:

- Processed species, subspecies and cultivar
- Moment of the growing period and sampled plant part
- Location of origin
- Climatic conditions during harvest
- Type of material (fresh / dry) subjected to processing.

The pharmaceutical / aromatherapeutic quality is not met by any of the oils studied and varies from year to year, depending on the above-mentioned parameters. Marketed volatile oils also fail to come under the limits set by the European Pharmacopoeia required to certify pharmaceutical quality. Therefore, the volatile oil producer should analyze each batch produced industrially, in order to be able to issue an appropriate test report.

The polyphenolic fraction varies qualitatively and quantitatively from:

- Processed species, subspecies and cultivar
- Climatic conditions during harvest
- Type of solvent used for extraction.

The antioxidant activity of the polyphenolic fraction varies with:

- Content of polyphenols present in extract, determined by species and subspecies
- Cultivar, samples from Bălteni (*L. Angustifolia* Mill.) being more active than cultivars collected from varieties cultivated in Balteni de Dambovita.

Antimicrobial and antifungal activity vary depending on the botanical provenance of the volatile oil sample:

- LHB has the best antibacterial and antifungal activity
- LHB has synergistic action with the tested antibiotics and antifungal substance in case the combination is applied
- All tested oils are active against *Bacillus Cereus* but have low anti-staphylococcal activity
- LAM is strongly antibacterial.

#### **Degree of originality and research perspectives**

Project's originality firstly derives from the investigation of certain new species and varieties of *Lavandula* cultivated under pedo-climatic conditions specific to the South-East of Romania.

Another element of originality consists in total pharmaco-botanical, chemical and biological issues used to explore the species under study.

The project contributes to wider phytochemical, phyto-pharmacological knowledge and correlation existing between pedoclimatic conditions in our country and chemical composition, substantiating the antimicrobial and antioxidant activity of species.

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