

Seasonal Variations in the Composition of the Essential Oils of *Lavandula angustifolia* (Lamiaceae)

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Seasonal variations in the composition of the essential oils obtained from the same individual (of the same genotype) of *Lavandula angustifolia* cultivated in Belgrade were determined by GC and GC/MS. The main constituents were 1,8-cineole (7.1-48.4%), linalool (0.1-38.7%), borneol (10.9-27.7%), β -phellandrene (0.5-21.2%) and camphor (1.5-15.8%). Cluster analysis showed that the 21 samples collected each month during the vegetation cycle were separable into three main clades with different compositions of essential oils. In the shoots with flowers, inflorescences and fruits of clade I, linalool is dominant, in the young leaves before flowering and old leaves of clade II, 1,8-cineole is dominant. In the young and incompletely developed leaves of clade III, β -phellandrene is dominant. The composition of the essential oils of lavender depended on the plant part and the stage of development.

Keywords: *Lavandula angustifolia*, Essential oil, Seasonal variations, Chemotypes.

Lavender (*Lavandula angustifolia* Mill., syn. *L. officinalis* Chaix, *L. spica* L., *L. vera* DC.) is a perennial woody shrub, native to the Mediterranean, and southwest and south Europe (Spain, France, Andorra, Italy, Croatia), and naturalized in other parts of Europe (Bulgaria, Greece, Russia) and north Africa [1]. As an ornamental, medical or industrial plant lavender is commercially cultivated in France, Spain, Portugal, Hungary, the UK, Croatia, Macedonia, Bulgaria, Australia, India, China and the USA.

Lavender oil is known for its excellent flavor, strong antibacterial, antifungal, spasmolytic, sedative, carminative, stomachic and diuretic effects. The plant is widely used in modern and traditional medicine, in perfumery and cosmetics, and as an insect repellent [2-4].

The chemical composition of lavender oil has been the object of many studies. Variations in the composition of the oils are influenced by the geographic origin of the plants, habitat types, harvesting time and drying method [5-8]. Commercially used essential oil is obtained from the flowering tops by steam distillation [9]. Generally, the data for lavender oil relate mainly to the quality of essential oils isolated from the flowers [5,8], while information on the composition of the essential oil of the leaves is rare [10]. The aim of this study was to describe seasonal variations in the composition of lavender essential oil. We report the results of the oil composition of 21 accessions of lavender, isolated from different plant parts of the same individual/genotype in different months during one vegetation season.

The oil yields of the tested samples were usually very similar (0.2-1.1%, with one sample 8.1%); the fruits yielded the highest amounts of oil. In the essential oils monoterpenoids were the most abundant (81.8%-99.2%). Monoterpene hydrocarbons were dominant only in the young leaves (43.0-47.5%), while in the other samples oxygenated monoterpenes dominated (60.1%-91.5%). Seventy-

eight components were identified, representing 100% of the oil. The main components were 1,8 cineole (7.1-48.4%), linalool (0.1-38.7%), borneol (10.9-27.7%), β -phellandrene (0.6-21.2%) and camphor (1.5-15.8%) (Table 1). These five components were dominant in all accessions and represented more than 40% of the total oils {in the young incompletely developed leaves (“yl” 42.7-44.8%), in the old completely developed leaves (“ol” 66.9-82.0%), in the inflorescences (“fl” 62.1-69.2%), and in the fruits (“fr” 75.4%)}.

Literature data for lavender oils relate mainly to the quality of the flower essential oils. Regardless of the geographical origin, habitat type and extraction methods, the dominant components of lavender essential oil isolated from flowers were linalool (23.3-43.4%) and linalyl acetate (20.2-39.6%) [5-8]. Our samples of flower oil also contained high concentrations of linalool (28.1-36.0%), while the amounts of linalyl acetate were very low (0-3.3%).

In the previously reported essential oil of lavender flowers grown near Belgrade, the main compounds were linalool (20.0%), camphor (19.9%) and linalyl acetate (12.5%) [11]. Although the plant that we analyzed was grown in almost identical climatic conditions, we found significant differences in the concentrations of these components (Table 1). These results indicate that the origin of the plant is a very important factor influencing the quality of the essential oil of flowers of lavender.

On the other hand our results for the essential oils isolated from the leaves coincide with those of Guittona *et al.* [10]; borneol and 1,8-cineole were detected in all leaf samples, while linalool and linalyl acetate were undetectable. The results of the cluster analysis performed on the basis of Pearson distances have shown a differentiation into three main clades, related to specific phases in the life-cycle of lavender. The first clade corresponded with the samples of reproductive organs – flowers and fruits (Clade I – ‘fl’

Table 1: Yield and chemical composition of the essential oils of *Lavandula angustifolia* samples.

SamplesLavAng	Clade III		Clade IIa				Clade IIb										Clade I					
	001	076	086	094	002	077	087	110	117	004	020	016	055	060	132	070	074	003	095	211	121	
	Apr yl a	Apr yl b	May yl 1	May yl 2	Apr ol a	Apr ol b	May ol	Aug. ol	Sep ol	Oct ol	Oct 30 ol	Nov ol	Dec ol	Jan ol	Jan 270 ol	Feb ol	Mar ol	Jun fl a	Jun fl b	Jun fl c	Sep fl	
Yield of oils (%)	0.2	0.6	0.2	0.4	0.5	0.3	0.2	0.3	0.3	0.4	1.1	0.2	0.2	0.4	0.6	0.2	0.4	0.5	0.5	0.8	8.1	
Constituents (%)																						
Tricyclene	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0
α -Thujene	0.4	0.3	0.3	0.2	0.0	0.0	0.1	0.2	0.1	0.3	0.1	0.7	0.0	0.0	0.0	0.1	0.0	0.3	0.1	0.2	0.1	0.1
α -Pinene	1.7	1.6	1.7	1.4	1.0	0.6	0.4	2.1	1.5	2.7	1.6	1.1	1.4	1.1	0.6	1.3	1.0	1.7	0.8	1.0	0.7	0.7
Camphene	0.5	0.4	0.7	0.6	1.1	0.6	0.7	0.8	0.7	1.0	0.9	1.1	1.0	0.9	0.6	1.0	1.0	0.7	0.5	0.5	0.5	0.5
Thuja-2,4(10)-diene	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.7	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sabinene	1.0	0.8	0.9	0.7	0.2	0.2	0.1	0.9	0.6	0.9	0.5	0.4	0.3	0.3	0.0	0.3	0.3	0.4	0.4	0.5	0.3	0.3
β -Pinene	2.3	2.1	2.5	2.1	1.3	0.9	0.6	3.4	2.6	4.3	2.5	1.1	1.7	1.7	0.8	1.7	1.4	2.1	1.4	1.4	1.5	1.5
Myrcene	2.8	2.5	1.9	1.4	0.0	0.4	0.2	1.3	0.9	1.2	0.1	0.3	0.3	0.1	0.1	0.1	0.1	1.5	0.8	1.1	0.3	0.3
α -Phellandrene	0.3	0.3	0.0	0.1	0.0	0.1	0.1	0.3	0.3	0.3	0.0	0.4	0.1	0.0	0.0	0.0	0.0	0.2	0.1	0.2	0.0	0.0
δ -3-Carene	5.5	5.5	4.2	2.6	0.2	1.2	0.5	3.7	3.1	4.5	0.5	0.5	0.7	0.5	0.0	0.4	0.2	1.1	1.2	0.9	0.5	0.5
α -Terpinene	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.2	0.0	0.0
<i>p</i> -Cymene	0.3	0.2	0.4	0.2	0.6	0.5	0.6	0.3	0.3	0.5	0.6	0.5	0.7	0.5	0.5	0.6	0.6	0.0	0.1	0.1	0.0	0.0
<i>o</i> -Cymene	0.7	0.4	1.0	0.5	1.6	1.2	1.5	0.9	0.9	1.3	1.4	2.1	1.8	1.3	1.4	1.5	1.6	0.3	0.5	0.3	0.4	0.4
β-Phellandrene	21.2	16.8	11.0	6.2	0.6	3.0	0.9	4.7	3.4	4.6	1.7	1.6	1.3	1.0	0.5	0.9	0.8	2.4	2.5	3.2	0.7	0.7
1,8-Cineole	10.6	9.4	20.5	21.9	48.4	32.4	37.1	35.0	33.3	37.7	37.7	24.6	34.8	39.0	38.4	43.2	42.3	7.1	18.1	15.9	17.2	17.2
<i>cis</i> - β -Ocimene	7.5	7.6	3.2	5.3	0.0	1.2	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	9.4	5.4	7.1	0.5	0.5
<i>trans</i> - β -Ocimene	2.8	3.5	1.0	1.2	0.0	0.6	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.2	0.6	1.0	0.2	0.2
γ -Terpinene	0.1	0.6	0.6	0.7	0.1	0.8	0.8	0.1	0.0	0.2	0.1	0.4	0.1	1.0	0.1	0.8	0.9	0.5	0.6	0.3	0.1	0.1
<i>cis</i> -Sabinene hydrate	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.1	1.0	0.0	0.0	0.0	0.0	0.0	0.6	0.0	0.0	0.1	0.0	0.4	1.0	1.0
<i>cis</i> -Linalool oxide (furanoid)	0.2	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.7	0.9	0.4	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.1	2.0	2.0
<i>p</i> -Mentha-2,4(8)-diene	0.4	0.4	0.2	0.2	0.3	0.2	0.2	0.3	0.3	0.3	0.2	0.0	0.2	0.3	0.2	0.3	0.1	0.0	0.1	0.0	0.0	0.0
Terpinolene	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.2	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.5	1.9	1.9
<i>trans</i> -Linalool oxide (furanoid)	0.2	0.3	0.0	0.2	0.1	0.2	0.2	0.0	0.0	0.2	0.1	0.0	0.2	0.0	0.0	0.0	0.0	0.8	0.2	0.0	0.0	0.0
<i>trans</i> -Sabinene hydrate	0.2	0.0	0.0	0.0	0.0	0.0	0.3	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.1	0.1
Linalool	0.4	0.4	0.6	11.9	0.3	0.3	0.4	0.1	0.3	0.3	0.8	0.3	0.3	0.4	0.1	0.4	0.4	36.0	28.1	31.6	38.7	38.7
α -Pinene oxide	0.0	0.0	0.0	0.0	0.0	0.2	0.1	0.1	0.1	0.0	0.1	0.0	0.0	0.1	0.1	0.1	0.1	0.0	0.0	0.1	0.0	0.0
<i>cis</i> - <i>p</i> -Menth-2-en-1-ol	0.2	0.0	0.2	0.1	0.3	0.2	0.2	0.2	0.1	0.2	0.3	0.0	0.2	0.2	0.3	0.2	0.2	0.0	0.1	0.1	0.0	0.0
α -Campholenal	0.1	0.0	0.0	0.0	0.2	0.2	0.2	0.1	0.1	0.2	0.2	0.4	0.3	0.2	0.2	0.2	0.2	0.1	0.1	0.2	0.0	0.0
<i>cis</i> -Limonene oxide	0.1	0.0	0.0	0.0	0.1	0.0	0.1	0.1	0.0	0.1	0.2	0.0	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0
<i>trans</i> -Pinocarveol	0.2	0.0	0.6	0.4	0.0	1.0	1.2	0.5	0.5	0.0	0.0	0.0	0.0	1.2	1.6	1.3	1.4	0.0	0.2	0.2	0.6	0.6
<i>trans</i> - <i>p</i> -Menth-2-en-1-ol	0.2	0.0	0.0	0.0	1.3	0.0	0.0	0.0	0.0	0.7	1.3	0.8	1.2	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0
Camphor	1.7	1.5	4.8	5.2	14.2	12.0	15.8	8.9	11.2	8.9	10.8	14.0	12.4	13.1	14.9	13.9	13.6	3.7	5.2	4.1	4.8	4.8
<i>trans</i> -Verbenol	0.3	0.4	0.1	0.0	0.2	0.1	0.2	0.2	0.2	0.3	0.5	0.0	0.2	0.2	0.4	0.2	0.2	0.0	0.1	0.0	0.0	0.0
Borneol	10.9	14.6	22.3	20.9	18.5	24.8	25.2	21.6	22.5	15.4	19.3	27.7	22.2	23.9	24.7	20.7	21.8	12.9	14.8	14.4	14.0	14.0
Terpinen-4-ol	0.8	0.0	1.1	2.2	1.4	0.9	1.5	1.1	1.3	1.0	1.5	0.4	1.0	1.1	1.1	1.4	1.3	7.1	4.0	4.8	6.7	6.7
<i>p</i> -Cymen-8-ol	0.1	0.0	0.3	0.3	0.5	0.4	0.7	0.4	0.4	0.3	0.6	0.0	0.3	0.4	0.5	0.5	0.5	0.9	0.2	0.1	0.0	0.0
Cryptone	2.5	1.1	2.1	0.7	0.7	0.4	0.6	0.0	0.6	0.8	1.6	0.5	0.5	0.5	0.8	0.5	0.6	0.0	0.9	0.6	1.5	1.5
α -Terpineol	0.8	1.3	1.3	2.0	0.5	0.6	0.5	2.0	1.7	1.0	0.9	0.0	0.7	0.7	0.5	0.7	0.7	2.8	2.0	2.4	1.5	1.5
Myrtenal	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.8	0.9	0.0	0.0	0.0	0.0	0.0	1.3	0.0	0.0	0.0	0.0	0.0	0.3	0.3
Myrtenol	0.3	0.0	0.6	0.4	1.0	1.1	1.0	0.1	0.1	0.7	1.3	1.2	1.3	1.2	0.0	1.2	1.2	0.1	0.4	0.0	0.0	0.0
<i>cis</i> -Piperitol	0.3	0.3	0.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0
<i>trans</i> -Piperitol	0.2	0.3	0.3	0.2	0.1	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Linalylformate	0.1	0.0	0.4	0.0	0.3	0.5	0.5	0.0	0.0	0.1	1.1	0.0	0.8	0.7	0.0	0.4	0.6	0.0	0.1	0.1	0.0	0.0
<i>trans</i> -Carveol	0.2	0.0	0.2	0.1	0.2	0.2	0.3	0.1	0.3	0.1	0.4	0.0	0.2	0.2	0.5	0.2	0.3	0.0	0.1	0.0	0.0	0.0
Nerol	0.2	0.0	0.2	0.0	0.1	0.0	0.0	0.2	0.2	0.1	0.3	0.9	0.2	0.1	0.3	0.1	0.1	0.2	0.2	0.0	0.0	0.0
Isobornylformate	0.0	0.0	0.5	0.6	0.7	0.8	1.0	0.4	0.4	0.5	0.7	0.0	0.8	0.7	0.8	0.7	0.7	0.6	0.5	0.4	0.9	0.9
Cumin aldehyde	0.5	0.0	0.4	0.1	0.3	0.3	0.4	0.2	0.3	0.3	0.5	0.0	0.0	0.2	0.4	0.3	0.3	0.1	0.2	0.0	0.0	0.0
Carvone	0.2	0.0	0.3	0.0	0.4	0.4	0.4	0.2	0.2	0.3	0.5	0.0	0.7	0.4	0.6	0.4	0.4	0.0	0.1	0.0	0.0	0.0
Car-3-en-2-one	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.4	0.0	0.0	0.0	0.0
Piperitone	0.2	0.0	0.0	0.0	0.2	0.0	0.0	0.0														

1,10-di- <i>epi</i> -Cubanol	0.3	0.4	0.2	0.0	0.0	0.3	0.0	0.2	0.2	0.1	0.3	0.3	0.4	0.2	0.1	0.2	0.0	0.0	0.0	0.0	
<i>t</i> -Cadinol	3.6	6.4	2.7	0.9	0.4	3.0	1.2	2.1	2.6	2.2	2.1	5.5	2.9	1.8	1.3	1.1	1.2	0.1	0.7	0.1	0.0
<i>α</i> -Cadinol	0.2	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.8	0.0	0.1	0.3	0.1	0.1	0.0	0.0	0.0	0.0
<i>α</i> -Bisabolol	0.2	0.3	0.9	1.4	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.1	0.6	0.0	0.0
Shyobunol	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>cis</i> -14-nor-MuuroI-5-en-4-one	0.9	2.6	0.8	0.4	0.0	0.8	0.1	0.0	0.0	0.3	0.2	0.0	0.4	0.2	0.2	0.1	0.0	0.0	0.2	0.5	0.0
Isobicyclogermacrene	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.2	0.0	0.3	0.2	0.3	0.0	0.0	0.0	0.0	0.0	0.0
Monoterpenoids	88.0	81.8	89.7	93.9	97.9	89.4	95.0	93.3	91.8	92.5	92.7	83.3	89.0	93.4	93.9	95.9	95.2	98.1	95.7	97.9	99.2
Hydrocarbons	47.5	43.0	29.6	23.4	7.1	11.5	7.2	19.5	15.0	22.1	10.4	11.3	9.6	8.7	5.0	9.1	8.1	22.0	15.1	18.5	7.7
Oxygenated monoterpenes	40.5	39.8	60.1	70.5	90.8	77.9	87.8	73.8	76.8	70.4	82.3	72.0	79.4	84.7	88.9	86.8	87.1	76.1	80.6	79.4	91.5
Sesquiterpenoids	11.6	18.2	10.3	5.9	2.0	10.6	5.1	6.9	8.1	7.7	7.2	16.5	11.1	6.7	6.0	4.5	5.1	1.7	4.5	1.8	0.7
Hydrocarbons	3.1	6.0	2.1	2.2	0.2	2.1	0.7	2.8	2.7	3.0	1.3	5.2	1.7	0.9	0.6	0.6	0.5	1.3	2.1	0.7	0.3
Oxygenated sesquiterpenes	8.5	12.2	8.2	3.7	1.8	8.5	4.4	4.1	5.4	4.7	5.9	11.3	9.4	5.8	5.4	3.9	4.6	0.4	2.4	1.1	0.4
Other	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	-	0.1	0.1

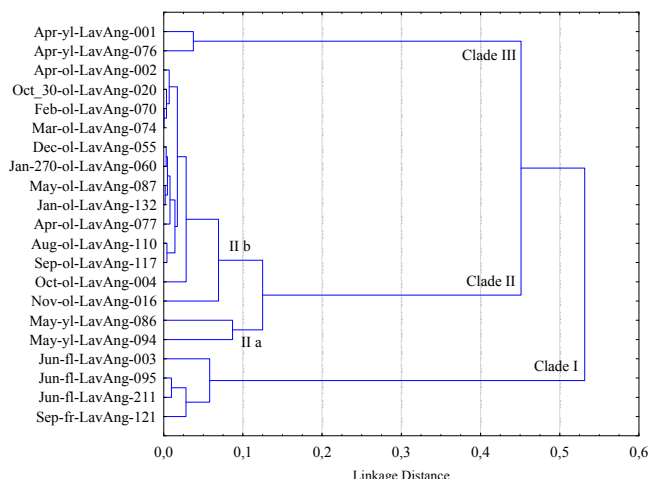


Figure 1: Cluster analysis of the chemical composition of the essential oils of 21 accessions of *Lavandula angustifolia* based on Pearson distances.

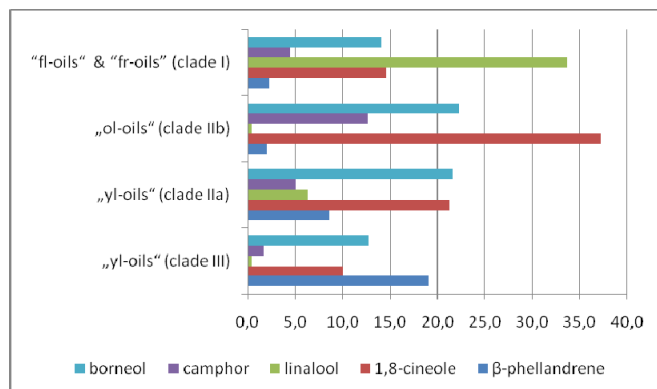


Figure 2: Dominant components of the essential oils of the basic clades in *Lavandula angustifolia*.

and ‘fr’), the second with the samples of young leaves from May and old leaves (Clade II – ‘yl’ and ‘ol’), and the third with samples of young leaves from April (Clade III – ‘yl’). In the second clade, the young leaves in the phase before flowering (Clade IIa – ‘yl’ May) were separated from the old leaves (Clade IIb – ‘ol’ Aug-Apr) (Figure 1). The results of the cluster analysis have shown three separated main clades related to different composition of essential oils (Figure 2). In the reproductive organs (‘fl’ and ‘fr’- Clade I) linalool is dominant and these oils can be classified as linalool chemotype. In the young shoots before flowering (‘yl’ May – Clade IIa) 1,8-cineole and borneol are the main compounds and these oils can be classified as 1,8-cineole/borneol chemotype. In the old shoots (‘ol’ – Clade IIb) 1,8-cineole is dominant and these oils can be classified as 1,8-cineole chemotype. Finally, in the young shoots

(‘yl’ April- Clade III), β-phellandrene is dominant and these oils can be classified as β-phellandrene chemotype.

Seasonal changes in the concentration of the dominant components of the essential oils of *L. angustifolia* are shown in Figure 3. Concentrations of 1,8-cineole and β-phellandrene showed opposite trends in the seasonal changes. The lowest percentages of 1,8-cineole were recorded in the young incompletely developed leaves (April – 10%). The concentration increased in the young leaves (May – 21.2%). In the old leaves (from August to May) the concentration of 1,8-cineole was uniform, and oscillated almost correctly in the range from 24.6% to 43.2%. 1,8-Cineole had a maximum concentration of 48.4% in the oil of the old overwintered leaves (April_ol). On the contrary, β-phellandrene had the largest concentration in the young, incompletely developed leaves (mean 19.0%), and during leaf development its concentration declined. Therefore, in the old leaves from August to May, concentrations of β-phellandrene were lower than 5.0%. Concentrations of camphor and borneol more or less increased from the young incompletely developed leaves (April_yl) to the old overwintered leaves (April_ol).

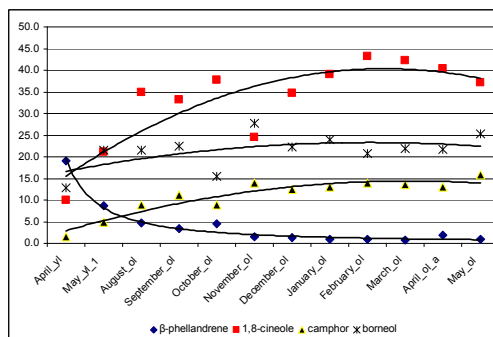


Figure 3: Seasonal changes in the concentration of the dominant components of essential oils of *Lavandula angustifolia*

Our results showed that the chemical composition of the essential oil of *L. angustifolia* depended on the stage of development. In the oil isolated from the young, incompletely developed leaves (‘yl’ April) β-phellandrene is dominant. In the next month the concentration of β-phellandrene decreased and the amount of 1,8-cineole increased. In the old leaves the concentration of 1,8-cineole was constantly high, especially in the oil of overwintered leaves. In the reproductive organs such as inflorescences and fruits linalool was the main compound, while the concentrations of β-phellandrene and 1,8-cineole were low.

Experimental

Plant material: The seasonal dynamic of the composition of the essential oils of cultivated lavender from Belgrade (Serbia) was

investigated. Accessions were collected in every month from April 2008 to May 2009. Accessions were classified into 4 groups: (i) 'yl-oils' – stem with 'young' incompletely developed leaves, (ii) 'ol-oils' – stem with 'old' completely developed leaves, (iii) 'fl-oils' – flowering stem and (iv) 'fr-oils' - only fruits. To avoid the influence of genetic and environmental factors, all analyzes were made from the same shrub, present 20 years in a private garden.

Essential oil isolation: Essential oils were isolated from fresh plant material, except for accession Lav-ang 020, which was distilled after 30 days and Lav-ang 132 after 270 days of drying at room temperature. The same distillation apparatus was always used under the same conditions. The essential oils were isolated by hydrodistillation for 2h, according to the standard procedure reported in the Sixth European Pharmacopoeia [9], using a Clevenger type apparatus. Oil samples were dissolved in ethanol and analyzed by GC/FID and GC/MS. Chemical analyses (GC/FID and GC/MS) used to identify and quantify essential oil constituents were accomplished using sharply defined and always the same analytical conditions.

Analytical gas chromatography (GC/FID): GC/FID analysis of the oils was carried out on a HP-5890 Series II GC apparatus (Hewlett-Packard, Waldbronn (Germany)), equipped with a split-splitless injector and an automatic liquid sampler (ALS), attached to a HP-5 column (25 m × 0.32 mm, 0.52 μm film thickness) and fitted to a flame ionization detector (FID). The carrier gas flow rate (H₂) was 1 mL/min, split ratio 1:30, injector temperature 250°C, detector temperature 300°C, while column temperature was linearly programmed from 40-260°C (at a rate of 4°C/min). Solutions of essential oil samples in ethanol (~1%) were consecutively injected by ALS (1 μL, split mode). Area percent reports, obtained as a result of standard processing of chromatograms, were used as the basis for the quantification purposes.

Gas chromatography/mass spectrometry (GC/MS): The same analytical conditions as those mentioned for GC/FID were employed for GC/MS analysis, along with a HP-5MS (30 m × 0.25 mm, 0.25 μm film thickness) column, using a HP G 1800C Series II GCD system (Hewlett-Packard, Palo Alto, CA (USA)). Instead of hydrogen, helium was used as carrier gas. The transfer line was heated at 260°C. Mass spectra were acquired in EI mode (70 eV), in the range m/z 40-450. Sample solutions in ethanol (~1 %) were injected by ALS (200 nL, split mode). The components of the oil were identified by comparison of their mass spectra with those from Wiley 275 and NIST/NBS libraries, using different search engines. The experimental values for retention indices were determined by the use of calibrated Automated Mass Spectral Deconvolution and Identification System software (AMDIS ver.2.1.), and compared with those from available literature [12] and used as an additional tool to support the MS findings.

Statistical analysis: Statistical analysis was performed in two steps: in the first, multivariate analysis was used in order to measure the distances between groups. These analyses were performed on complete data sets. The UPGMA (unweighted pair-group average linkage) clustering method based on Pearson distances was used to measure the similarities between each measured unit. In the second step, the seasonal dynamics of individual components of the essential oils were examined. These analyses were performed on (i) 'yl-oils' – stem with 'young' incompletely developed leaves and (ii) 'ol-oils' – stem with 'old' completely developed leaves. Statistical analyses were performed with the package Statistica 5.1 (STATSOFT 1996) and scatterplots with trendlines using the package Excel for Windows 97. The emergence of new young shoots in April is designated as the beginning of the season in which the trends of changes in the composition of essential oils were analyzed.

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