IN VITRO PROPAGATION AFFECTS THE COMPOSITION OF NARROW-LEAVED LAVENDER ESSENTIAL OILS

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The aim of this paper was to identify and determine using GC/FID and GC/MS method of volatile compounds of essential oils obtained from three varieties of narrow-leaved lavender grown in the field and in vitro cultures. The essential oils were isolated by hydrodistillation in Deryng apparatus. It was found that the analyzed essential oils varied in terms of chemical composition depending on the variety and conditions of growth. 64 to 87 different compounds were identified in oils. Essential oils of all 3 varieties obtained in in vitro cultures contained large amounts of borneol (13.38 – 32.17 %). This compound was also dominant in plants obtained from in vivo conditions in varieties 'Ellagance Purple' (11.32 %) and 'Blue River' (13.36 %) and in the '*Munstead*' variety the dominant compound was linalool (12.67 %). High concentration of epi-α-cadinol (9.85 - 20.18 %) was found in essential oils obtained from in vitro cultured plants. Globulol was found in high concentration (9.95 %) in the 'Munstead' variety grown in in vitro conditions. However, significant quantitative and qualitative differences were found on composition of essential oils obtained from plants grown in the field and in vitro conditions. There was a lack of (*E*)-β-ocimene, 3-octyn-2-one, 1-octen-3-yl acetate, sabina ketone, pinocarvone, trans-carveol, nerol, epi-longipinanol or humulene epoxide II. In comparison to oils obtained from filed-grown plants, the oils isolated from plants grown in in vitro conditions contained the less volatile compounds identified in the final stage of GC/FID and GC/MS analysis, i.e. thymol, carvacrol, γ-gurjunene, trans-calamene, α-calacorene, khusinol, 8-cedren-13-ol.

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Introduction

The lavender genus (*Lavandula*) belongs to the mint family (*Lamiaceae Lindl.*). It comprises 39 species, numerous hybrids and approximately 400 registered varieties.[1] Lavandula is native to the Mediterranean region and is commercially grown, among others, in France, Spain, the United Kingdom, Bulgaria, Australia, China and in the United States.[2] The most commonly grown and best-known species of the Lavandula genus are *Lavandula stoechas*, *Lavandula dentata* and most of all *Lavandula angustifolia*.

Narrow-leaved lavender (*Lavandula angustifolia* Mill. syn. *Lavandula officinalis Chaix*) is used in many industries mainly due to its essential oils characterized by a specific aroma. The oils are used in perfume[3,4] and cosmetics industry.[5,6] Apart from the aroma, the oils show a number of valuable medicinal properties including anti-bacterial properties and are, therefore, used in medicine and pharmaceutical industry[3,7-10] Essential oils are mixtures of mainly monoterpene and sesquiterpene compounds, however, their composition depends on various factors connected with both biological material used for isolating the oil as well as with the physical factors of this technological process.[11-13]

Essential oils are contained in the secretory tissue covering the entire above-ground portion of the plant, therefore essential oil can be isolated from flowers,[14-16] stem[17] or leaves.[18] However, the type of material used for oil isolation affects the concentration of particular compounds of essential oils.[19] The method of oil isolation also has a significant effect on its composition. Reverchon and Della Porta[20] isolated essential oils using two methods: hydrodistillation (steam distillation – the most widely-used commercial method), and supercritical fluid extraction. The analysis of the composition of the obtained oils showed significant differences of, among others, linalyl acetate – using supercritical extraction its concentration was 34.7%, and only 12.1 % using hydrodistillation.

Numerous studies point to significant variations in terms of the composition of essential oils isolated from species of the *Lavandula genus*. Linalool, camphor and 1,8-cineole were the main components of essential oil isolated from *L. latifolia* Med.[21] and *L.*×*intermedia* Emeric ex Loiseleur.[22] The main components of oil obtained from *L. pedunculata* (Miller) Cav.[23] were camphor and 1,8-cineole[23] and in the case of *L. pinnata* L. – α- and β-phellandrene.[24] In essential oil of *L. viridis* L'Hér, 1,8-cineole and camphor were the main components[25] and in oil obtained from *L. stoechas* L. – fenchon, camphor, myrtenyl acetate and 1,8-cineole.[26] Main compounds dominating the aroma of essential oil isolated from *L. angustifolia* are linalool which amounts to 25 – 38 %, and linalool acetate 25 – 45%.[27-29] The studies were limited to filed-grown plants or growing in natural conditions.

The method of plant tissue culture allows for the quick proliferation of tissue in controlled, sterile conditions. So far, it has been used in the production of a large number of plant cuttings genetically identical to the mother plant.[30,31] Nowadays, as the technique of in vitro culture develops and its costs decrease, the aim is to apply this technique for the proliferation of plant tissue to obtain secondary metabolite, including essential oils. However, for this to happen it is necessary to determine the influence of the conditions of in vitro plant cultures on the variations in the amount and

composition of essential oils – as was found in *Thymus caespititius,*[32] *Achillea millefolium,*[33] *Agastache rugosa*[34] and *Lantana camara*.[35]

At present, there is a little information on the composition of essential oils isolated from tissues of plants of Lavandula genus, proliferated in in vitro cultures. The composition of isolated essential oils was identified as for, among others, *L. pedunculate,*[15,23] *L.viridis,*[25] *L. vera* and *L. viridis.*[36] The study by Prasad et al.[37] identified the effects of the proliferation of shoots of *L. officinalis syn., L. angustifolia* var. Sher-e-Kashmir in in vitro cultures by comparing the composition of essential oils obtained from the mother plant with that obtained from clones which were previously proliferated in in vitro cultures and later grown in the field. However, there is a shortage of publications on the quantitative and qualitative composition of essential oils obtained from tissues of *Lavandula angustifolia* during proliferation in in vitro cultures. The present study aims to provide the qualitative and quantitative analysis of the composition of essential oils obtained from three varieties of narrow-leaved lavender 'Ellagance Purple', 'Blue River' and 'Munstead' grown in natural conditions as mother plants as well as those proliferated in in vitro conditions.

Materials and methods

Field - grown plant

The material used in the study was field - grown plants of narrow-leaved lavender (*Lavandula angustifolia L.*) of three varieties: 'Ellagance Purple', 'Blue River' and 'Munstead' and cultured in in vitro conditions. Field - growing plants were obtained from experimental cultivation by the Department of Horticulture of the West Pomeranian University of Technology in Szczecin conducted in the period 2013–2014. The seeds for initiation of the culture were obtained from voucher specimen number 195 from the Institute of Natural Fibres and Medicinal Plants in Poznan, Poland. The fragments of stems without inflorescence, harvested in mid-July from plants at full bloom, were used to initiate in vitro culture and air dried to obtain a sample for isolation of essential oil.

In vitro plants

The fragments of shots of the aforementioned field - growing plants were used as original explants for the initiation of in vitro cultures. The explants – 1 cm long fragments of shoots were placed on MS media of mineral composition according to Murashige and Skoog[38] supplemented with vitamins: nicotinic acid 0.5 mg·dm^{-3}, pyridoxine·HCl 0.5 mg·dm-3, thiamine·HCl 0.1 mg·dm^{-3}, glycine 2 mg·dm^{-3} and 8 g·dm^{-3} agar, 30 g·dm^{-3} sucrose and 100 mg·dm^{-3} inositol. pH was adjusted to 5.7 with solutions of 0.1 M NaOH and HCl prior to autoclaving. Media were sterilized by autoclaving at 121 °C for 20 min at 1.1 atm. The sterile shoots induced to grow were proliferated on the media with a mineral composition according to Murashige and Skoog[38] supplemented with 2 mg·dm^{-3} kinetin and 0.2 mg·dm^{-3} indole-3-acetic acid. Proliferation cycle was repeated 4 times after 6 weeks each. The cultures were placed in phytotrons at 23±10C with a 16 h light/8 h night photoperiod with a PPFD of 30 µmol·m^{-2}·s^{-1} supplied by 21 W cool white fluorescent lamps. Then the proliferated lavender shoots (together with leaves) were air dried and constituted a sample for isolation of essential oils.

Gas chromatography with mass spectroscopy (GC/MS) of essential oils

The analysis was conducted in Central Agroecology Laboratory of the University of Life Sciences in Lublin. Dried material in weight of 20 g of the field - grown and in vitro plant tissue were used for the purpose of oil isolation. Isolation of essential oils was repeated in three replicates. The percentage content of essential oil was determined using hydrodistillation method of steam distillation in Deryng apparatus, according to European Pharmocopoeia.[39] The obtained results of the assays were statistically analyzed using analysis of variance. For two-way cross-classification, evaluating the significance of differences with Tukey's confidence intervals and performing least significant difference (LSD) calculations at the level of significance $\alpha = 0.05$.

The chemical constituents of the essential oil were analyzed by capillary gas chromatography (GC/FID) and gas chromatography-mass spectrometry (GC/MS). The oil was stored at 4 °C until the GC/FID and GC/MS analysis. The qualitative and quantitative composition of essential oil was determined by GC/MS method using gas chromatograph Varian Chrompack CP-3800 equipped with a with a mass detector (4000 GC-MS/MS) and a flame ionization detector (FID). A VF-5ms column (an equivalent of DB-5) was used. The carrier gas was Helium (He) with constant flow rate 0.5 ml min^{-1}. The temperature of the dispenser was 250 ºC, split 1:100. The dosing was 1 µl of the solution (10 µl of sample in 1000 µl of hexane). The temperature gradient was applied (50 °C for 1 min, then increase to 250 °C at a rate 4 °C min^{-1}, 250 °C for 10 min). The range recorded was 40–1000 m/z, the scan rate 0.8 sec scan^{-1}. Kovats retention index was determined on the grounds of a series of alkanes C_{10}-C_{40}.

The HP Chemstation software was used for the collecting and processing the data. The qualitative analysis was based on the identification of compounds in samples by comparing MS spectra with standard spectra of NIST Mass Spectral Library[40] and with data available in the literature.[41] (Adams 2001). The compounds which showed conformity of mass spectra with the standard library spectra of more than 95% were taken into account. The relative percentage content of the analyzed compounds was based on the peak area of the total ionic current of all the compounds present in a given sample. The quantitative composition of essential oil was determined assuming that the sum of individual compounds amounts to 100%. The analysis was repeated in three replicates for each experiment. The obtained results of the assays were statistically analyzed using analysis of variance for 1-way cross-classification, separately for each compound and variety, evaluating the significance of differences with Tukey's confidence intervals and performing least significant difference (LSD) calculations at the level of significance $\alpha = 0.05$.

Results and discussion

From dry plant material of field - grown narrow - leaved lavender, 0.53% of the essential oil was obtained from 'Ellagance Purple', 0.52% from 'Blue River' and 0.90% from the 'Munstead' variety. Propagation of plants in in vitro cultures resulted in a decrease in the content of essential oil in shoots and leaves – distillation efficacy was 0.51% for 'Ellagance Purple', 0.20% 'Blue River' and 0.84% for the 'Munstead' variety (Table 1).

Table 1. Hydrodistillation efficacy of *Lavandula angustifolia* varieties field - grown and propagated in vitro. The values represent the means of three replicates ± S.E.

Cultivar (A)	**Plant type (B)**		**Mean**
	field-grown	***in vitro***	
'Ellagance Purple'	0.53 ± 0.03	0.51 ± 0.04	0.52
'Blue River'	0.52 ± 0.03	0.20 ± 0.01	0.36
'Munstead'	0.90 ± 0.04	0.84 ± 0.04	0.87
Mean	0.65	0.52	
LSD 0.05 for:			
Cultivar (A)	0.180		
Plant type (B)	0.120		
Interaction (AxB)	0.254		
Interaction BxA	0.216		

Table 2 show the detailed composition and amounts of particular compounds identified in essential oils of 'Ellagance Purple', 'Blue River' and 'Munstead' varieties of narrow-leaved lavender grown in in vivo and in-vitro conditions. The GC/MS analysis allowed for the identification of 92.44-97.71 % of compounds in the analyzed essential oils. Most of the compounds belong to monoterpenoids group and monoterpenoid esters. The chemical composition of essential oils isolated from shoots of the three varieties of narrow-leaved lavender field and in vitro grown varied greatly (Table 2). In the oil isolated from the field - grown plant, 83 compounds were identified in 'Ellagance Purple', 87 in 'Blue River' and 82 in the 'Munstead' variety. In comparison, the number of compounds identified in the essential oils isolated from in vitro plants was smaller – 72 in 'Ellagance Purple', 69 in 'Blue River' and 64 in the 'Munstead' variety. A decrease in the number of constituent compounds in essential oils of *Caryopteris clandonensis* proliferated in vitro was also found by Łuczkiewicz et al.[42] According to Avato et al.,[43] the decrease in the number of compounds produced is connected with juvenility of plant tissue in in vitro conditions which are associated with a drop in production of more complex metabolites produced in the subsequent stages of metabolic pathways.

In essential oils isolated from all 3 varieties of field - grown plants, the dominant compounds were: borneol (from 9.32 % in 'Munstead' variety to 13.36 % in 'Blue River'), linalool (from 3.71 % in 'Blue River' to 12.67 % in 'Munstead'), and globulol (from 4.40 % in 'Blue River' to 6.85 % in 'Ellagance Purple' variety). Daferera et al.[44] isolated essential oil of a slightly different concentration from narrow-leaved lavender grown in natural conditions. The authors found a high concentration of linalool (44.5 %), linalyl acetate (32.7 %) and 1.8-cineole (4.8 %), which were identified to be the main compounds out of 8 identified and constituted 82 % of the total composition of the oil. The analysis of the composition was made with the use of GC/MS, and Lickens-Nickerson method was used for isolation of essential oil applying distillation with organic solvents lighter than water. The analysis of the composition of essential oils obtained from seven varieties of narrow-leaved lavender: 'Jubileina', 'Hemus', 'Hebar', 'Raya', 'Sevtopolis', 'Drujba' and 'Karlovo' using GC/MS method was done by Zagorcheva et al.[16] The plants were harvested in summer and isolation of oil was done from fresh flowers using steam distillation. In the course of the study, 32 compounds were identified, with linalool having the highest concentration (18.74-34.43 %), followed by linalyl acetate (20.68-32.72 %), lavandulyl acetate (2.47-7 %) and caryophyllene (1-3.83 %). However, in presented study, those compounds were in lower concentration. The study on oils isolated from flowers of *Lavandula angustifolia* Mill. by Wesołowska et al.[45] shows the highest concentration of linalool (28.78–30.68 %), linalool acetate (12.35–17.67 %) and α-terpineol (7.57–11.49 %) among the identified compounds (depending on the variety from 43 to 47). 29 compounds present in lavender essential oils were found by Daferera et al.[46] with linalool 25.5 %, linalyl acetate 17.7 %, and α-terpineol 5.6 %. However, according to Adaszyńska et al.[47] essential oil of narrow-leaved lavender of 'Munstead', 'Munstead Strain', 'Lavender Lady', 'Ellagance Purple', 'Blue River' varieties contained linalool (23.9–15.8 %), linalyl anthranilate (12.3–1.6 %), 1-terpinen-4-ol (9.7–5.5 %), terpineol (p-menth-1-en-8-ol) (7.9–4.0 %) and linalool oxide (4.7–1.1 %). From 18 to 21 different compounds were identified with GC/MS analysis of the essential oils. According to Cong et al.,[48] 17 different compounds comprise lavender essential oil isolated from *Lavandula angustifolia*. The highest concentration was found for linalool (44.54 %), geraniol (11.02 %), lavandulol acetate (10.78 %), 3.7-dimethylo-2,6-octadien-1-ol (10.35 %) and izoterpineol (6.75 %). Own research conducted by the authors of the present paper show a high concentration of geraniol (12.28 %) in essential oils isolated from the field - grown lavender of 'Munstead' variety.

The dominant compound in terms of the composition of essential oil isolated from lavender plants proliferated in in vitro conditions, similarly to the oil isolated from field - grown lavender plants, was borneol – 32.17 % in 'Ellagance Purple', 25.75 % in 'Blue River' and 13.38 % in the 'Munstead' variety. However, there were some significant quantitative and qualitative differences in the composition of oils isolated from filed-grown plants and in vitro. Linalyl isobutanoate, one of the main compounds present in oils isolated from field - grown plants in the concentration from 5.12 to 9.77 %, was not found in oils isolated from plants grown in vitro regardless of analysed variety – similarly to other compounds, such as (*E*)-β-ocimene, cis-linalool oxide, 3-octyn-2-one, 1-octen-3-yl acetate, sabina ketone, trans-carveol, 4-methylene-isophorone, nerol, epi-longipinanol or humulene epoxide II. Sudria et al.[49,50] studied the effect of conditions of culturing on the production of essential oils by *L. dentata* and found that the variation in the amount of oil produced in in vitro cultures, as well as its concentration, is connected with the addition of plant growth regulators to proliferation media, which affects the endogenous regulation of metabolic pathways.

Table 2. Essential oil composition (%) of varieties *Lavandula angustifolia* isolated from field-growing parent plants and the respective in vitro shoot cultures. The values represent the means of three replicates ± S.E.

Compound	RT, min	KI	'Ellagance Purple'		'Blue River'		'Munstead'	
			field - grown	*in vitro*	field - grown	*in vitro*	field - grown	*in vitro*
tricycylene	6.838	922	tr.	tr.	0.10^{ns}	0.14±0.15^{ns}	0.11^{a}	0.05^{b}
α-thujene	6.921	925	0.11^{b}	0.23^{a}±0.02	0.21^{ns}	0.73±0.84^{ns}	0.17^{b}	0.36±0.01^{a}
α-pinene	7.157	933	1.67^{b}±0.00	2.69^{a}±0.17	1.17±0.00^{ns}	1.33±0.45^{ns}	0.88±0.03^{b}	3.25±0.02^{a}
α-fenchene	7.620	947	0.07		0.07			
camphene	7.673	949	0.52^{b}	1.21±0.07^{a}	1.37±0.06^{ns}	0.98±1.19^{ns}	1.67±0.09^{a}	1.19±0.00^{b}
thuja-2.4-(10)-diene	7.797	953	tr.	tr.	tr.	tr.	tr.	tr.
benzaldehyde	8.090	963	tr.		tr.		tr.	
verbenene	8.341	971	0.60±0.07 ^{a}	0.28±0.07^{b}	0.64±0.04^{a}	0.36±0.11^{b}	0.33±0.02^{b}	0.58±0.01^{a}
sabinene	8.384	972	0.26±0.04 ^{b}	0.98±0.02^{a}	0.27±0.02^{a}	0.09±0.03^{b}	0.12^{b}	0.87±0.01^{a}
β-pinene	8.560	978	5.17±0.06^{b}	6.50±0.04^{a}	2.11^{a}	0.29±0.09^{b}	0.83^{b}	6.26±0.01^{a}
3-octanone	8.777	985	0.19±0.26		0.27±0.01		0.34±0.02	
myrcene	8.901	989	0.07^{b}	0.34±0.01^{a}	0.16±0.02^{b}	0.55±0.17^{a}	0.58^{ns}	0.37±0.16^{ns}
dehydro-1.8-cineole	8.949	990	0.20^{a}	0.10±0.01^{b}	0.13±0.01			
3-octanol	9.161	997			tr.		tr.	
trans-isolimonene	9.250	1000				0.08±0.02		0.15
α-phellandrene	9.498	1004		tr.				
δ-2-carene	9.573	1005	0.78^{b}	2.74±0.19^{a}	1.29±0.02^{b}	4.49±1.52^{a}	0.66±0.03^{b}	4.47±0.01^{a}
α-terpinene	9.850	1009	0.06^{b}	0.07^{a}	tr.		0.06^{b}	0.11±0.00^{a}
p-cymene	9.945	1010	1.29±0.01^{a}	0.82±0.04^{b}	1.47±0.02^{a}	0.91±0.27^{b}	0.71^{b}	1.14±0.01^{a}
o-cymene	10.118	1012	2.40±0.04^{a}	1.31±0.08^{b}	3.11±0.05^{a}	1.21±0.36^{b}	1.62±0.07^{b}	1.80±0.03^{b}
sylvetrene	10.278	1015	1.65±0.03^{b}	2.17±0.10^{a}	3.79±0.03^{ns}	2.64±0.82^{ns}	2.41±0.10^{a}	2.05±0.03^{b}
1.8-cineole	10.389	1016	4.04±0.05^{b}	4.84±0.27^{a}	5.48^{a}	0.86±0.09^{b}	2.70±0.07^{a}	2.00^{b}
(Z)-β-ocimene	10.485	1018	0.19±0.01	tr.	0.10^{a}	0.05±0.01^{b}	1.08±0.04	tr.--
(E)-β-ocimene	10.852	1023	0.12		0.11		0.73±0.03	
o-cresol	11.064	1026	tr.		tr.		tr.	
γ-terpinene	11.289	1029	0.14^{ns}	0.14^{ns}	0.14±0.01^{ns}	0.09±0.03^{ns}	0.18^{ns}	0.20^{ns}
trans-linalool oxide	11.716	1035	0.96±0.01		0.59±0.01		0.47	
cis-sabinene hydrate	11.724	1035		0.21±0.01	tr.	0.09±0.03		0.13±0.01
m-cymenene	12.111	1041	0.10±0.02		0.11±0.01		0.06	
terpinolene	12.130	1042		0.14±0.01		0.27±0.07		0.24±0.00
p-mentha-2.4(8)-diene	12.269	1043		0.24±0.01		0.20±0.06		0.22
cis-linalool oxide	12.280	1043	0.67±0.04		0.44±0.03		0.43±0.02	
p-cymenene	12.463	1046	0.27±0.06^{a}	0.12±0.01^{b}	0.21±0.04^{a}	0.11±0.03^{b}	0.13^{b}	0.15^{a}
linalool	12.755	1050	5.94±0.26^{a}	2.32±0.05^{b}	3.71^{ns}	3.31±0.88^{ns}	12.67^{a}	0.36±0.00^{b}
trans-sabinene hydrate	12.817	1051		tr.				tr.
3-octyn-2-one	12.881	1052	0.25		0.10±0.01		0.09	
1-octen-3-yl acetate	13.001	1053	0.09		0.42±0.01		0.50	
endo-fenchol	13.485	1060		0.05				

trans-mentha-2.8-dien-1-ol	13.557	1061	0.06		0.23±0.01		0.11±0.00	
trans-p-mentha-2.8-dien-1-ol	13.559	1062		0.13		0.25±0.08		0.13
cis-menth-2-en-1-ol	13.645	1063	0.12		0.42±0.02		0.21±0.00	
α-campholenal	13.742	1064	0.29±0.01[a]	0.06[b]	0.22±0.02		0.12±0.02[ns]	0.12[ns]
cis-limonene oxide	13.951	1067	0.21[a]	0.11[b]	0.28±0.09[a]	0.09±0.03[b]	0.09[b]	0.15±0.00[a]
cis-p-mentha-2.8-dien-1-ol	14.086	1069	0.11±0.01[b]	0.15[a]	0.26±0.02[ns]	0.28±0.07[ns]	0.12[b]	0.15[a]
nopinone	14.193	1070	0.46		0.19			
trans-pincarveol	14.253	1071	2.01±0.04[a]	1.59±0.03[b]	1.04±0.20[a]	0.10±0.03[b]	0.45[b]	1.58±0.00[a]
trans-verbenol	14.420	1074	0.68±0.02		0.40		0.25±0.00	
cis-verbenol	14.428	1074		0.66		0.35±0.09		0.72±0.03
camphor	14.487	1074	1.30±0.01[b]	1.79±0.04[a]	1.89±0.16[a]	1.04±0.21[b]	1.33±0.14[a]	0.62±0.00[b]
sabina ketone	14.854	1080	0.11		0.09±0.01		tr.	
pinocarvone	15.030	1082	2.64±0.02		1.63±0.01		0.72	
borneol	15.393	1087	11.32±0.02[b]	32.17±0.89[a]	13.36±0.06[b]	25.75±5.41[a]	9.32±0.28[b]	13.38±0.07[a]
p-cymen-8-ol	15.666	1091	2.05±0.04[a]	1.50±0.01[b]	2.01±0.08[a]	1.10±0.22[b]	4.22±0.17[a]	2.02±0.00[b]
cryptone	15.900	1095	2.17±0.04[a]	0.81±0.03[b]	5.12±0.11[a]	1.01±0.18[b]	2.84±0.08[a]	0.88±0.13[b]
α-terpineol	16.217	1099	3.20±0.00[a]	2.87±0.07[b]	1.91[a]	0.56±0.09[b]	2.61±0.10[a]	2.38±0.00[b]
myrtenal	16.622	1110	0.25±0.01[b]	0.57±0.01[a]	0.21±0.12[b]	0.68±0.10[a]	0.12±0.03[b]	1.22[a]
verbenone	16.724	1113	1.29±0.03[a]	0.30±0.01[b]	1.50±0.02[a]	0.21±0.06[b]	0.61±0.04[a]	0.43±0.01[b]
trans-carveol	17.009	1121	0.47		0.76±0.02		0.37±0.02	
4-methylene-isophorone	17.069	1123	0.15		0.26±0.02		0.13	
nerol	17.165	1126	tr.		0.23		0.40	
isobornyl formate	17.342	1131	0.60		0.68		0.45±0.02	
cis-sabinene hydrate acetate	17.357	1131		0.30±0.01		0.15±0.04		0.14±0.01
cis-carveol	17.477	1135		0.08		0.07±0.02		
cumin aldehyde	17.837	1146	0.81±0.03		3.71±0.02		1.96±0.05	
carvone	17.885	1147	0.49±0.16[ns]	0.31±0.01[ns]		0.35±0.10		0.30±0.17
geraniol	18.067	1152	1.04±0.04[a]	0.18±0.02[b]	0.64±0.03[a]	0.27[b]	12.28±0.44	
piperitone	18.235	1157			0.21±0.01		0.09	
tymoquinone	18.689	1171	0.33		0.36±0.01		0.10±0.13	
linalyl acetate	19.177	1185	0.97±0.08[a]	0.12±0.01[b]	1.68±0.02[a]	0.43±0.04[b]	0.85±0.04[a]	0.49[b]±0.10
neo-isopulegyl acetate	19.247	1187	0.06±0.07		0.73±0.01		1.37±0.02	
iso-3-thujyl acetate	19.251	1187		0.05		0.39±0.04		0.09
α-terpinen-7-al	19.345	1190	tr.		0.14		0.07±0.00	
p-cymen-7-ol	19.512	1195	0.56		1.61±0.01		0.80±0.03	
thymol	19.521	1196		0.12		0.28±0.02		0.27±0.01
carvacrol	19.756	1202		0.13		0.10±0.03		0.21±0.00
perilla alcohol	19.778	1203	0.25		0.21±0.07		0.10±0.01	
myrtenyl acetate	20.761	1232	0.08		0.07			
3- oxo-p-menth-1-en-7-al	21.062	1241	0.44		0.73±0.01		0.36±0.02	
neryl acetate	21.684	1260	0.24[b]	3.77±0.21[a]	0.31[b]	1.10±0.02[a]	0.70±0.04[b]	1.56±0.07[a]
linalyl isobutanoate	22.346	1280	9.77±0.18		6.07±0.09		5.12±0.20	

longicyclene	22.466	1283			0.07			
α-funebrene	23.135	1304				0.07±0.02		
α-cis-bergamotene	23.480	1316		tr.		0.22--		0.07
E-caryophyllene	23.665	1322	1.62±0.01[a]	1.31±0.05[b]	2.05±0.02[b]	4.39±0.08[a]	0.94±0.05[b]	3.54±0.17[a]
β-cedrene	23.870	1330		0.07		0.09±0.07		0.22±0.01
α-trans-bergamotene	24.104	1338	0.17		0.25		0.10±0.00	
coumarin	24.259	1343	0.09		0.08±0.03		0.12±0.00	
aromadendrene	24.458	1350	0.09		0.13		0.06	
β-duprezianene	24.460	1351		tr.		0.24±0.02		
epi-β-santalene	24.552	1354			0.10		tr.-	
β-copaene	24.558	1355		tr.		0.27±0.02		
(Z)-β-farnesene	24.739	1360		tr.		0.39±0.03	0.38	
α-himachalene	24.898	1366		0.10±0.03		0.36±0.01		
trans-muurola-3.5-diene	25.048	1371		0.09		0.43±0.02		0.12±0.01
dehydro-aromadendrane	25.182	1376		0.06±0.01		0.11±0.03		0.07
γ-amorphene	26.628	1392			0.10±0.04		0.11±0.07	
9-epi-(E)-caryophyllene	25.720	1395		tr.		0.21±0.02		
γ-gurjunene	26.341	1415		0.12±0.01		0.53±0.09		0.18±0.01
β-bisabolene	26.432	1417					0.13±0.00	
α-amorphene	26.447	1418		3.60±0.23		0.14±0.04		0.15±0.01
γ-cadinene	26.621	1423	1.90±0.02		1.60±0.01[b]	8.38±1.01[a]	1.40±0.04[b]	4.68±0.36[a]
trans-calamene	26.840	1430		0.24±0.02		0.62±0.11		0.30±0.03
epi-longipinanol	27.149	1439	0.45		0.44		0.32±0.02	
α-calacorene	27.473	1449		0.11±0.01		0.26±0.07		0.16±0.01
caryophyllene oxide	27.772	1458	0.67±0.01[a]	0.16±0.08[b]	0.42±0.02[a]	0.11±0.03[b]	0.42±0.03[b]	0.95±0.06[a]
globulol	28.704	1487	6.85±0.01[a]	2.07±0.23[b]	4.40±0.03[a]	1.58±0.54[b]	4.62±0.08[b]	9.95±0.88[a]
khusimone	29.418	1511	0.14±0.01[ns]	0.12±0.01[ns]	0.10±0.01[ns]	0.17±0.06[ns]	0.10[ns]	0.15[ns]
humulene epoxide II	29.509	1515	0.15±0.02		0.08±0.01		0.09	
cubenol	29.656	1521	0.80±0.03[ns]	0.97±0.12[ns]	0.59±0.03[b]	1.85±0.73[a]	0.61±0.04[b]	1.44±0.04[a]
epi-α-cadinol	30.449	1551	7.45±0.53[ns]	9.85±1.49[ns]	5.87±0.14[ns]	20.18±7.30[ns]	7.05±0.01[b]	15.81±0.55[a]
epoxyallo-alloaromadendrene	30.610	1559	0.31±0.18		0.18±0.01			
himachalol	30.800	1566	0.42±0.00[a]	0.17±0.06[b]	0.30±0.02[ns]	0.41±0.23[ns]	0.30±0.00[b]	0.47±0.08[a]
14-hydroxy-9-epi-(E)-caryophyllene	31.218	1576	0.52±0.02[a]	0.19±0.04[b]	0.24±0.03[ns]	0.37±0.24[ns]	1.68±2.79[ns]	0.37±0.02[ns]
cis-14-nor-murol-5-en-4-one	31.634	1599	1.03±0.04		0.76±0.02		0.80±0.00	
14-hydroxy-α-muurolene	32.368	1624	±0.02		0.29±0.02		0.24±0.01	
khusinol	32.384	1624		0.19±0.03		0.42±0.33		0.40±0.01
8-cedren-13-ol	32.810	1639		tr.		0.10±0.09		0.09
nootkatone	33.149	1650	0.63±0.02[a]	0.29±0.05[b]	0.42±0.01[ns]	0.52±0.40[ns]	0.44±0.01[b]	0.53±0.03[a]
Total identified compounds			83	72	87	69	82	64

Total identified (%)	96.03	94.98	95.20	95.76	97.71	92.44
monoterpene hydrocarbons	14.87	19.70	15.79	14.16	12.00	22.88
oxygenated monoterpenes	57.56	55.57	61.02	39.18	65.92	29.91
sesquiterpene hydrocarbons	3.86	5.70	4.30	16.71	3.12	9.49
oxygenated sesquiterpenes	19.74	14.01	14.09	25.71	16.67	30.16

RT (min) - retention index on VF-5ms capillary column; KI - Kovats index was determined on grounds of a series of alkanes C_{10}-C_{40}; [a] - higher value, [b] - lower value, [ns] - not statistically significant; tr. - trace <0.05 % or 0.001 mg mL^{-1}; -- - not detected

In comparison to the oils isolated from the field - grown plants, the oils isolated from plants grown in in vitro conditions are characterised by the presence of the less volatile compounds, identified in the final stage of GC/MS analysis, i.e. thymol, carvacrol, epoxy allo-alloaromadendrene, khusinol, 8-cedren-13-ol, trans-calamene. γ-amorphene was found in trace quantity in essential oils of 'Munstead' (0.11 %) and 'Blue River' (0.10 %) variety obtained from in vivo plants, whereas in 'Ellagance Purple' variety and in oils obtained from in vitro plants of the same varieties the compound was not present. There was an increase in the concentration of γ-cadinene to 4.68 % for 'Munstead' and 8.38 % for 'Blue River' variety. Additionally, α-amorphene was found in the 'Ellagance Purple' variety of in vitro grown plant in the amount of 3.6 %. Epi-α-cadinol, a compound which was found in all essential oils, was identified in substantial quantity in in vitro oils. 9.85 % in oil of 'Ellagance Purple', 20.18 % in 'Blue River' and 15.81 % in the 'Munstead' variety. The concentration of globulol is also noteworthy as it was identified in all isolated essential oils with the highest concentration (9.95 %) in in vitro 'Munstead' essential oil, however in lower concentration in 'Ellagance Purple' (2.07 %) and in 'Blue River' (1.58 %) variety. Other compounds which were found only in plants propagated in in vitro cultures were, among others, terpinolene, p-mentha-2.4(8)-diene, trans-p-mentha-2.8-dien-1-ol, cis-verbenol, cis-sabinene hydrate acetate, iso-3-thujyl acetate.

Zuzarte et al.[15] isolated essential oils from the field - grown plant and from in vitro cultures of *L. pendunculata*, classified as belonging to two chemotypes: 1,8-cineole/camphor and fenchone. The GC/MS analysis showed that the main components of the oils were the same for the field - grown as well as in vitro propagated plants, however, their concentration varied. Higher concentration of compounds in plants propagated in in vitro cultures classified as 1,8-cineole/camphor chemotype was found for, among others, α-pinene (13.6 %) and bornyl acetate (10.4 %); in terms of the fenchone chemotype – α-pinen (10.2 %) and camphor (11.6 %). The chemical uniformity of essential oils isolated from the field - grown plants and in vitro shoot cultures propagated on MS media supplemented with 0.5 $mg \cdot dm^{-3}$ BAP and micropropagated plants of the same clone *L. viridis* was also observed by Nogueira and Romano.[25] In all analyzed oils, among 45 identified compounds, the same main compounds were determined. Monoterpene fraction identified in oils isolated from in vitro culture showed a slight variation in terms of the content of carbohydrate and oxidized components in comparison to oil obtained from the mother plant.

Conclusion

In vitro method of propagating plant tissues allow for obtaining the large bulk of plants in a relatively short period of time, yet the method can affect the metabolism of plants and, consequently, the qualitative and quantitative composition of produced essential oils. In turn, this can affect the aroma, and even modify the antioxidative and antimicrobial action of the essential oils. The antimicrobial and antioxidant activity of lavender essential oils isolated from the field -grown plants are confirmed. However, there are not yet published results in respect to lavender essential oils isolated from *in vitro* plants. Presented results have shown that *in vitro* conditions leads to a change biochemical profile of the essential oils and increasing the concentration e.g. borneol, γ-cadinene, epi-α-cadinol or emergence of others chemical compounds. That might have an impact on differences in the antimicrobial and antioxidant activity of essential oils. Our previous research shows that essential oils isolated from *in vitro* propagated plants show higher antioxidant and antimicrobial activity especially with respect to bacteria presented on the human skin, in comparison with oils isolated from the field - grown plants. Essential oils with confirmed and more than average antioxidant and antimicrobial potential could be used in cosmetic industry as a natural preservative, which would extend cosmetics durability without the addition of synthetic preservatives.

References

[1]Upson T., Andrews S.,. The genus *Lavandula*. (1st ed.). Timber Press, Inc., USA, **2004.**

[2]Cavanagh, H. M.A., Wilkinson, J. M., Lavender essential oil: a review. *Aust. Infect. Control*., **2005**, *10*, 35-38. https://doi.org/10.1071/HI05035

[3]Cavanagh, H. M. A., Wilkinson. J. M., Biological activities of lavender essential oil. *Phytother. Res*., **2002**, *16*, 301-308. https://doi.org/10.1002/ptr.1103

[4]Lahlou, M.,. Essential oils and fragrance compounds: bioactivity and mechanisms of action. *Flavour Fragr. J*. **2004**, *19*, 159-165. https://doi.org/10.1002/ffj.1288

[5]Aburjai, T., Natsheh, F. M., Plants used in cosmetics. *Phytother. Res*., **2003**, *17*, 987-1000. https://doi.org/10.1002/ptr.1363

[6]Baumann, L. S., Less-known botanical cosmeceuticals. *Dermatol. Ther*., **2007**, *20*, 330-342. https://doi.org/10.1111/j.1529-8019.2007.00147.x

[7]Adam, K., Sivropoulou, A., Kokkini, S., Lanaras, T., Arsenakis, M., Antifungal activities of *Origanum vulgare* subsp. *hirtum, Mentha spicata, Lavandula angustifolia,* and *Salvia fruticosa* essential oils against human pathogenic fungi, *J. Agric. Food Chem.*, **1998**, *45*, 1739-1745. https://doi.org/10.1021/jf9708296

[8]Hui, L., He, L., Huan, L., Xiao, Lan L., Ai, Guo Z., Chemical composition of lavender essential oil and its antioxidant activity and inhibition against rhinitis related bacteria. *Afr. J. Microbiol. Res.*, **2010**, *4*, 309-313.

[9]Lis-Balchin, M., Deans, S. G., Eaglesham, E., Relationship between bioactivity and chemical composition of commercial essential oils. *Flavour Fragr. J.*, **1998**, *13*, 98-103. https://doi.org/10.1002/(SICI)1099-1026(199803/04)13:2<98::AID-FFJ705>3.0.CO;2-B

[10]Lis-Balchin, M., Hart, S., Studies on the mode of action of the essential oil of Lavender (*Lavandula angustifolia* P. Miller). *Phytother. Res.*, **1999**, *13*, 540-542.

[11]Landmann, C., Fink, B., Festner, M., Dregus, M., Engel, K. H., Schwab, W., Cloning and functional characterization of three terpene synthases from lavender (*Lavandula angustifolia*). *Arch. Biochem. Biophys*., **2007**, *465*, 417-429. https://doi.org/10.1016/j.abb.2007.06.011

[12]Van de Braak, S. A. A. J., Leijten, G. C. J. J., *Essential Oils and Oleoresins: A Survey in the Netherlands and other Major Markets in the European Union*. CBI, Centre for the Promotion of Imports from Developing Countries, Rotterdam. **1999**, 116.

[13]Zheljazkov, V. D., Cantrell, C. L., Astatkie, T., Jeliazkova, E., Distillation time effect on lavender essential oil yield and composition. *J. Oleo Sci.*, **2013**, *62*, 195-199. https://doi.org/10.5650/jos.62.195

[14]Lane, A., Boecklemann, A., Woronuk, G. N., Sarker, L., Mahmoud, S. S., A genomics resource for investigating the regulation of essential oil production in *Lavandula angustifolia*. *Planta*, **2010,** *231*, 835-845. https://doi.org/10.1007/s00425-009-1090-4

[15]Zuzarte, M., Dinis, A. M., Cavaleiro, C., Salgueiro, L. R., Canhoto, J. M., Trichomes, essential oils and *in vitro* propagation of *Lavandula pedunculata (Lamiaceae). Ind. Crop Prod.*, **2010**, *32*, 580-587. https://doi.org/10.1016/j.indcrop.2010.07.010

[16]Zagorcheva, T., Stanev, S., Rusanov, K., Atanassov, I., Comparative GC/MS analysis of lavender (*Lavandula angustifolia* Mill.) inflorescence and essential oil volatiles. *J. Agr. Sci. Technol.*, **2013**, *5*, 459-462.

[17]Adaszyńska-Skwirzyńska, M., Swarcewiczm M., Dobrowolskam A., The Potential of Use Lavender from Vegetable Waste as Effective Antibacterial and Sedative Agents. *Med. Chem.*, **2014**, *4*, 734-737.

[18]Lakušić, B., Lakušić, D., Ristić, M., Marčetić, M., Slavkovska, V. Seasonal variations in the composition of the essential oils of *Lavandula angustifolia* (*Lamiacae*). *Nat. Prod. Commun.*, **2014**, *9*, 859-862.

[19]Nadalin, V., Lepojević, Ž., Ristić, M., Vladić, J., Nikolovski, B., Adamović, D. Investigation of cultivated lavender (*Lavandula officinalis* L.) extraction and its extracts. *Chem. Ind. Chem. Eng. Quart.*, **2014**, *20*, 71-86. https://doi.org/10.2298/CICEQ120715103N

[20]Reverchon, E., Della Porta, G., Supercritical CO_2 Extraction and Fractionation of Lavender Essential Oil and Waxes. *J. Agric. Food Chem*., **1995**, *43*, 1654-1658. https://doi.org/10.1021/jf00054a045

[21]Munõz-Bertomeu, J., Arrillaga, I., Segura, J., Essential oil variation within and among natural populations of *Lavandula latifolia* and its relation to their ecological areas. *Biochem. Syst. Ecol.* **2007**, *35*, 479-488. https://doi.org/10.1016/j.bse.2007.03.006

[22]Desautels, A., Biswas, K., Lane, A., Boeckelmann, A., Mahmoud, S. S., Suppression of linalool acetate production in *Lavandula×intermedia*. *Nat. Prod. Commun*., **2009**, *4*, 1533-1536.

[23]Zuzarte, M., Gonçalves, M. J., Cavaleirom C., Dinism A. M., Canhotom J., Salgueiro, L., Chemical composition and antifungal activity of the essential oils of *Lavandula pedunculata* (Miller). *Cav. Chem. Biodivers*., **2009**, *6*, 1283-1292. https://doi.org/10.1002/cbdv.200800170

[24]Figueiredo, A. C., Barroso, J. G., Pedro, L. G., Sevinate-Pinto, I., Antunes, T., Fontinha, S. S., Looman, A., Scheffer, J. J. C., Composition of the essential oil of *Lavandula pinnata* L. var. *pinnata* grown on madeira. *Flavour Fragr. J*., **1995**, *10*, 93-96. https://doi.org/10.1002/ffj.2730100207

[25]Nogueira, J. M., Romano, A., Essential oils from micropropagated plants of *Lavandula viridis*. *Phytochem. Anal.*, **2002**, *13*, 4-7. https://doi.org/10.1002/pca.609

[26]Giray, E. S., Kirici, S., Kaya, D. A., Türk, M., Sönmez, O., Inan, M., Comparing the effect ofsub-critical water extraction with conventional extraction methods on the chemical composition of *Lavandula stoechas*. *Talanta*, **2008**, *74*, 930-935. https://doi.org/10.1016/j.talanta.2007.07.040

[27]Demissie, Z. A., Sarker, L. S., Mahmoud, S. S., Cloning and functional characterization of β-phellandrene synthase from *Lavandula angustifolia*. *Planta*, **2011**, *233*, 685-696. https://doi.org/10.1007/s00425-010-1332-5

[28]Da Porto, C., Decorti, D., Kikic, I., Flavour compounds of *Lavandula angustifolia* L. to use in food manufacturing: Comparison of three different extraction methods. *Food Chem.*, **2009**, *112*, 1072-1078. https://doi.org/10.1016/j.foodchem.2008.07.015

[29]D'Auria, F. D., Tecca, M., Strippoli, V., Salvatore, G., Battinelli, L., Mazzanti, G., Antifungal activity of *Lavandula angustifolia* essential oil against *Candida albicans* yeast and mycelial form, *Med. Mycol*., **2005**, *43*, 391-396. https://doi.org/10.1080/13693780400004810

[30]Al-Bakhit, A. A. M., Sawwan, J. S., Al-Mahmoud, M. S., *In vitro* Propagation of Two Lavandula Species: *Lavandula angustifolia* and *Lavandula latifolia L.* Medica. J., *J. Agr. Sci.* **2007**, *3*, 16-25.

[31]Falk, L., Biswas, K., Boeckelmann, A., Lane, A., Mahmoud, S. S., An Efficient Method for the Micropropagation of Lavenders: Regeneration of a Unique Mutant. *J. Essent. Oil Res*., **2009**, *21*, 225-228. https://doi.org/10.1080/10412905.2009.9700154

[32]Mendes, M. D., Figueiredo, A. C., Oliveira, M. M., Trindade, H., Essential oil production in shoot cultures versus field-grown plants of *Thymus caespititius. Plant Cell Tiss. Org.*, **2013**, *113*, 341-351.

[33]Alvarenga, I. C. A., Silva, S. T., Bertolucci, S. K.V., Pinto, J. E. B. P., Pacheco, F. V., Application of Thidiazuron (TDZ) for *in vitro* multiplication of yarrow (*Achillea millefolium* L.) and profile of volatile compounds. *Aust. J. Crop Sci*., **2015**, *9*, 948-953

[34]Zielińska, S., Piątczak, E., Kalemba, D., Matkowski, A., Influence of plant growth regulators on volatiles produced by in vitro grown shoots of *Agastache rugosa* (Fischer & C.A.Meyer) O. *Plant Cell Tiss. Org*., **2011**, *107*, 161-167.

[35]Affonso, V. R., Bizzo, H. R., De Lima, S. S., Esquibela, M. A., Sato, A. Solid phase microextraction (SPME) analysis of volatile compounds produced by *in vitro* shoots of *Lantana camara* L. under the influence of auxins and cytokinins. *J. Braz. Chem. Soc*., **2007,** *18*, 1504-1508. https://doi.org/10.1590/S0103-50532007000800009

[36]Gonçalves, S., Romano, A., *In vitro* culture of lavenders (*Lavandula* spp.) and the production of secondary metabolites. *Biotechnol. Adv*., **2013**, *31*, 166-174. https://doi.org/10.1016/j.biotechadv.2012.09.006

[37]Prasad, A., Shukla, S. P., Mathur, A., Chanotiya, C. S., Mathur, A. K., Genetic fidelity of long-term micropropagated *Lavandula officinalis* Chaix.: an important aromatic medicinal plant. *Plant Cell Tiss. Organ. Cult*., **2014,** *120*, 803-811. https://doi.org/10.1007/s11240-014-0637-7

[38]Murashige, T., Skoog, F., A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant*, **1962**, *15*, 473-497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

[39]European Pharmacopoeia. 4th ed. Version 4.08., Strasbourg: EDQM. **2004**, 3158-9.

[40]NIST Mass Spectral Library. NIST/EPA/NIH, Gaithersburg, MD, USA (**2002**)

[41]Adams, R. P., *Identification of Essential Oil Compounds by Gas Chromatography/Quadrupole Mass Spectroscopy*. Allured, Carol Stream, IL, USA. **2001**.

[42]Łuczkiewicz, M., Jesionek, A., Kokotkiewicz, A., Migas, P., Mardarowicz, M., Szreniawa-Sztajnert, A., Zabiegała, B., Buciński, A., Production of essential oils from in vitro cultures of *Caryopteris* species and comparison of their concentrations with *in vivo* plants. *Acta Physiol. Plant*., **2015**, *37*, 58. https://doi.org/10.1007/s11738-015-1804-0

[43]Avato, P., Fortunato, I. M., Ruta, C., D'Elia, R., Glandular hairs and essential oils in micropropagated plants of *Salvia officinalis* L. *Plant Science*, **2005**, *169*, 29-36. https://doi.org/10.1016/j.plantsci.2005.02.004

[44]Daferera, D. J., Ziogas, B. N., Polissiou, M. G., GC-MS Analysis of Essential Oils from Some Greek Aromatic Plants and Their Fungitoxicity on *Penicillium digitatum*. *J. Agric. Food Chem.*, **2000**, *48*, 2576-2581. https://doi.org/10.1021/jf990835x

[45]Wesołowska, A., Jadczak, D., Grzeszczuk, M., Influence of distillation time on the content and composition of essential oil isolated from lavender (*Lavandula angustifolia* Mill.). *Herba Polonica*, **2010**, *56*, 24-36.

[46]Daferera, D. J., Tarantilis, P. A., Polissiou, M. G., Characterization of Essential Oils from *Lamiaceae* Species by Fourier Transform Raman Spectroscopy. *J. Agric. Food Chem*., **2002**, *50*, 5503-5507. https://doi.org/10.1021/jf0203489

[47]Adaszyńska, M., Swarcewicz, M., Dzięcioł, M., Dobrowolska, A., Comparison of chemical composition and antibacterial activity of lavender varieties from Poland. Nat. Prod. Res., **2013**, *27*, 1497-1501. https://doi.org/10.1080/14786419.2012.724408

[48]Cong, Y., Abulizi, P., Zhi, L., Chemical composition of the essential oil from *Lavandula angustifolia* from Xinjiang, China. *Chem. Nat. Comp*., **2008**, *44*, 810. https://doi.org/10.1007/s10600-009-9210-8

[49]Sudria, C., Piñol, M. T., Palazon, J., Cusido, R. M., Vila, R., Morales, C., Bonfill, M., Cañigueral, S., Influence of plant growth regulators on the growth and essential oil content of cultured *Lavandula dentata* plantlets. *Plant Cell Tiss. Organ. Cult.*, **1999**, *58*, 177-184. https://doi.org/10.1023/A:1006377003962

[50]Sudria, C., Palazón, J., Cusid, R., Bonfill, M., Piñol, M. T., Morales, C., Effect of benzyladenine and indolebutyric acid on ultrastructure, glands formation, and essential oil accumulation in *Lavandula dentata* plantlets. *Biologia Plantarum*, **2001**, *44*, 1-6. https://doi.org/10.1023/A:1017998800224

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