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CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF LAVENDER (*Lavandula angustifolia* Mill.) ABOVEGROUND PARTS

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Abstract. Biochemical assessment was performed of leaves, flower buds and flowers of lavender. High positive correlation was demonstrated between the essential oil contents and antioxidant activity (AA) (R = 0.9688), total phenolic acids and AA (R = 0.9303), as well as high negative correlation between flavonoid contents and AA (R = -0.9760). Results of the foregoing studies also suggest that AA of lavender (77.5–86.3%) is more correlated with the essential oil and phenolic compound contents than with the contents of flavonoids, anthocyanins and tannins. The predominant compounds in the oil obtained from leaves were *epi-α*-cadinol (17.8%), cryptone (10.4%), 1,8-cineole (7.3%) and caryophyllene oxide (7.2%), and of the oil distilled from flowers: linalyl acetate (22.3–32.1%) and linalool (23.9–29.9%).

Key words: total phenolic acids, DPPH radical scavenging activity, essential oil constituents

INTRODUCTION

Medicinal oil plants are popular throughout the world, both on natural stands and as cultivated plants. Biosynthesis of volatile oils can take place in different plant organs and its course is determined by ontogenetic and environmental factors [Najfian et al. 2012, Zheljazkov et al. 2012]. Similarly, the synthesis and accumulation of other active substances, such as flavonoids, phenolic compounds, anthocyanins or tannins, so it is modified through the course of plant development [Shafaghat et al. 2012] and depends upon climatic factors. Medicinal lavender (*Lavandula angustifolia* Mill.) is an appreciated curative plant, used also in cosmetic industry, perfumery, food and for decorative purposes. The main active substance of lavender raw material is essential oil, accumu-

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lated in the amounts ranging from 1.13 to 9.25% [Jianu et al. 2013, Kara and Baydar 2013]. The lavender essential oil (LEO) composition depends on cultivar and growing conditions. Medicinal lavender from north eastern Iran accumulated in its inflorescences and leaves respectively: 6.25 and 0.64% of volatile oils [Hassanpouraghdam et al. 2011]. As predominant components of LEO the following are mentioned: linalool, linalool acetate and linalyl acetate [Stanojević et al. 2011, Najafian et al. 2012,], 1,8-cineole, borneol, fenchon and camphor [Afsharypuor and Azarbayejany 2006, Torabbegi and Aberoomand Azar 2013], as well as menthol and α-pinene [Rostami et al. 2012]. The share of above-mentioned components undergoes is subject to, among others, ontogenetic variability. Concentrations of 1,8-cineole, borneol and terpinen-4-ol in LEO was higher before blooming than in full blooming, quite contrary to the contents of linalool and linalool acetate [Najafian et al. 2012] It was also demonstrated that the manner of distillation may affect the chemical profile of EO [Zheljazkov et al. 2012, Torabbegi and Aberoomand Azar 2013].

The therapeutic properties of lavender mainly result from the activity of volatile substances. The LEO demonstrates antibacterial [Hussain et al. 2011a, Rostami et al. 2012, Danh et al. 2013], anti-fungal [Canvanagh and Wilkinson 2005], antiviral [Orhan et al. 2012], antioxidant [Hussain et al. 2011b, Hamad et al. 2013] and sedative activities [Huang et al. 2008]. Antimicrobial activity of LEO is quite difficult to assign the presence of a particular component [Shafaghat et al. 2012, Jianu et al. 2013]. However, antiviral activity of linalool, linalool oxide, linalool ester, borneol and eugenol against *Herpes simplex* was confirmed [Orhan et al. 2012]. The antioxidant activity of LEO, in turn, probably results from high concentration of linalool [Hamad et al. 2013]. Except the essential oil in lavender flowers there also contain other active substances, such as flavonoids, including anthocyanins, phenolic compounds, tannins, coumarins, phytosterols and mineral compounds. The most important feature of plant flavonoids and phenolic compounds is their antioxidative activity that brings about many pharmacological applications [Brunetti et al. 2013, Szwajgier et al. 2013]. There are reports confirming the effectiveness of flavonoids and phenolic compounds in preventing neoplasms [Roy et al. 2002, Soobrattee et al. 2006]. The antioxidant activity of plant extracts can be explained both by the mechanism of phenolic compound activity and by the effect of synergic activity of the above-mentioned compounds, as well as flavonoids [Eghdami and Sadeghi 2010, Nuñez et al. 2012, Saeed et al. 2012]. Considering the above relationships, the essential oil and phenolics content and their corresponding antioxidant potential of diverse organs (leaves, flower buds and flowers) were assayed.

MATERIALS AND METHODS

Plant material. Leaves, flower buds and flowers of medicinal lavender (*Lavandula angustifolia* Mill.) were collected from 2-year old plants grown in an experimental farm of the University of Life Sciences in Lublin in South-Eastern Poland (51°23'N, 22°56''E). In this area there is fawn soil, formed on loess sediment, with the contents of organic matter in the amount of 1.6%. The sowing material came from seed producing company PNOS Ożarów Mazowiecki. Lavender was grown in the spacing of 60 × 40 cm.

During vegetation period the indispensable cultivation procedures were performed (removal of dried sprouts, several procedures of hand-weeding) and feeding the plants twice with nitrogen in the form of ammonium saltpeter having 34% N (single dose of about 7 kg N ha^{-1}). Lavender leaves were collected before blooming (2.06.2013), flower buds and flowers – in the initial phase of their development (respectively: 14.06 and 15.07.2013). The collected plant material was dried in the temperature of 35°C. After drying, the leaves were characterized by light green hue and strong aroma, whereas buds and flowers had the characteristic blue-violet colour and a specific aroma.

Total flavonoids content. Flavonoids content were spectrophotometrically determined. 10 g of medium powdered raw material was weighed out (sieve 0.315 mm) into a round bottomed flask 20 ml acetone was added, as well as 2 ml of HCl (281 g l^{-1}), 1 ml of metenamine solution (5 g l^{-1}) and it was retained for 30 min in boiling state on water bath under a reflux condenser. The hydrolysate was filtered through cotton-wool into a 100 ml measuring flask, the precipitate with cotton wool was put in the flask and 20 ml of acetone was added and then it was kept in boiling state again for 10 minutes. Etching was repeated once again. The extracts were filtered into the same measuring flask and acetone was added to it. 20 ml of the solution was measured out into the distributor, 20 ml of water was added and extracted with ethyl acetate, by 15 ml portions and 3 times 10 ml. The connected organic layers were washed twice with 40 ml of water, filtered into a 50 ml measuring flask and topped up with ethyl acetate. Two samples were prepared for determination: to 10 ml of basic solution 2 ml of aluminum chloride solution (20 g l^{-1}) and it was topped up with a mixture (1:19) of acetic acid (1.02 kg l^{-1}) with methanol up to 25 ml. To prepare a comparative solution, 10 ml of basic solution was topped up with a mixture (1:19) of acetic acid (1.02 kg l^{-1}) with methanol up to 25 ml. After 45 min absorbance of the solutions was measured at 425 nm, applying the comparative solution as a reference. The total flavonoid content (%) was expressed according to the formula: $X = \frac{A \cdot k}{m}$, where: *A* means study solution absorbance, k – conversion factor for quercetin k = 0.875 ($a\frac{l\%}{lcm}$ = 714), m – raw material weighed sample in g.

Total phenolic acids content. To a 10 ml measuring test-tube 1.0 ml of water extract was weighed out, as well as 1 ml of hydrochloric acid (18 g l^{-1}), 1 ml of Arnov's reagent, 1 ml of sodium hydroxide (40 g l^{-1}) and that was topped up with water to 10 ml (solution A). Then the solution absorbance was measured at 490 nm, applying a mixture of reagents without the extract as reference. The contents of phenolic acids (%) was determined in conversion to coffee acid ($C_9H_2O_4$), assuming absorbability $a\frac{l\%}{lcm}$ = 285, according to the formula: $X = \frac{A \cdot 3.5087}{m}$, where A means absorbance of solution A, m – a weighted sample of raw material in g.

Anthocyanins content. 1 g of dried, previously comminuted and averaged raw material was weighed out. The weighed sample was quantitatively transferred to a measuring cylinder of the capacity of 250 cm^3, containing a mixture of HCl in methanol (850 ml of methanol and 150 ml of hydrochloric acid were poured into a 1000 ml meas-

uring flask, and the combined reagents were left there for 24 hours). The prepared maceral was left under the hood, tightly covered, for 24 hours. Then absorbance of the filtrate was measured at wave length $\lambda = 535$ nm.

Tannins content. Tannins were spectrophotometrically determined after they had been extracted from dried raw material. The determination was performed with protection against direct effect of light, applying water deprived of CO_2. 5 g of finely pulverized raw material (sieve diameter: 0.16 mm) was weighed out into a 250 ml measuring flask, 150 ml of water was added and it was kept for 30 min in a bath with boiling water. Then the mixture was cooled down with a stream of running water, quantitatively transferred into a 250 ml measuring flask, topped up with water and left for complete sedimentation of the raw material. The liquid from above the sediment through filter paper, first 50 ml were rejected, the remaining filtrate was used for determinations. To determine the total polyphenols content, 5.0 ml of filtrate was topped up with water, to 25.0 ml of that solution 1.0 ml of phosphoro-molybdenic-wolframic reagent was added, then 10.0 ml of water and that was topped up with a solution of sodium carbonate (290 g l^{-1}) to 25.0 ml. After 30 min absorbance was measured at 760 nm, applying water as a reference (A_1). To determine the contents of polyphenols not bounding with powder, to 10.0 ml of filtrate 0.10 g of hide powder was added, which had been fiercely shaken for 1 hour and then it was filtered. 5.0 ml of filtrate was topped up with water up to 25.0 ml, and then to 2.0 ml of that solution 1.0 ml of phosphoro-molybdenic-volframic reagent was added, then 10.0 ml of water and it was topped up with a sodium carbonate solution (290 g l^{-1}) to 25.0 ml. After 30 min absorbance was measured at 760 nm, applying water as a reference (A_2). A comparative solution was prepared: immediately before determination 50.0 mg pyrogalol was dissolved in water and topped up with water to 100.0 ml. 5 ml of the obtained solution was topped up with water to 100.0 ml, to 2.0 ml of that solution 1.0 ml of phosphoro-molybdenic-volframic reagent was added, as well as 10.0 ml of water and that was topped up with a sodium carbonate solution (290 g l^{-1}) to 25.0 ml. After 30 min absorbance was measured at 760 nm, applying water as a reference (A_3). The content of tannins (%) was calculated in conversion to pyrogalol ($C_6H_6O_3$), according to the formula: $X = \frac{62.5 \cdot (A_1 - A_2) \cdot m_2}{A_3 m_1}$, where A_1 means absorbance of polyphenols in the studied solution, A_2 – absorbance of polyphenols not binding with hide powder in the studied solution, A_3 – absorbance of the comparative solution of pyrogalol, m_1 – a weighed sample of raw material in g, m_2 – a weighed sample of pyrogalol in g.

DPPH radical scavenging activity assay. DPPH radical scavenging activity was expressed as % of DPPH inhibition. The determination was performed according to the method given by Yen and Chen [1995], and the calculation of DPPH inhibition according to the formula given by Rossi et al. [2003]: % DPPH = $100 - \left[\frac{A_t}{A_r} \cdot 100\right]$. To prepare a reagent containing a solution of radicals 0.012 g DPPH (2,2'-diphenyl-1-picrylhydrasyl) was weighed out, transferred to a measuring flask of the capacity of 100 ml, filled up with methanol (100%), then it was dissolved in an ultrasound washer for 15 min. The blind assay (A_r) was prepared as follows: 1 ml of distilled water was measured out into a test tube (pH > 5), as well as 3 ml of methanol (100%) and 1 ml of DPPH solution. Having stirred it, after 10 minutes it was read on a spectrophotometer at 517 nm,

against methanol (100%). To perform the examined assay (A_t) 1 ml of a sample was diluted in methanol and 3 ml of methanol (100%) was added, as well as 1 ml of DPPH solution. The sample was stirred and after 10 min it was read on a spectrophotometer at 517 nm, against methanol (100%).

Essential oil distillation. The dried plant material, after samples had been weighed out (20 g each) was placed in glass flasks of the capacity of 1 dm^3, poured over with 400 ml of water and designed for distillation conducted in Clevenger-type apparatuses for 3 hours, counting from the moment when the contents of flask started to boil and the first drop was distilled. The intensity of heating was regulated in such a way as to 3–4 ml of liquid flew into the receiver per one minute. After distillation had finished, cooling was switched on, the oil was led to micro-scale and after 30 min the result was read.

Essential oil composition. The quantitative and qualitative composition of lavender oil obtained from leaves, flower buds and developed flowers was determined with the use of gas chromatography and mass spectrometry methods (GC-MS) Varian 4000 MS/MS. For our studies we used the apparatus Varian 4000 MS/MS with VF-5 m column (an equivalent of DB-5), the registered range 40–1000 m/z, scanning speed 0.8 sec/scan. The carrier gas was helium, the steady flow; 0.5 ml min^{-1}. The temperature of batcher was 250°C, the temperature gradient of 50°C was applied for 1 min, then the increase to 250°C with the speed of 4°C min^{-1} and 250°C for 10 min. Split 1:1000 m/z, 1 μl of solution was dosed (10 μl of assay in 1000 μl of hexane). Non-isothermal Kovacs' retention indexes were determined on the basis of a range of alkanes C_{10}–C_{40}. The qualitative analysis was carried out on the basis of MS Spectral Library [2008]. The identity of the compounds was confirmed by their retention indices taken from the literature [Adams 2004] and our own data.

Statistical analysis. All the chemical analyses were performed in three repetitions. Significance of differences was assessed using Tukey's confidence intervals at the significance level alpha = 0.05. Correlation coefficients were calculated using the formulas given by Oktaba [1986], at the level of 0.05 and 0.01.

RESULTS AND DISCUSSION

Chemical composition and antioxidant activity of lavender leaves and flowers. The chemical composition of examined lavender leaves and flowers was differentiated and dependent upon the plant organ and stage of its development (tab. 1). Concentration of essential oil was on average 2.2 ml 100 g^{-1} and increased as lavender developed, from 0.6 ml 100 g^{-1} (leaves) through 2.7 ml 100 g^{-1} (flower buds) to 3.2 ml 100 g^{-1} (developed flowers). Similarly, as plant development progressed in the examined organs the contents of anthocyanins increased and in leaves it equaled 3.1 mg 100 g^{-1}, while in flowers it ranged from 4.3 to 9.9 mg 100 g^{-1} (respectively: flowers in the phase of buds and full development). The examined lavender flowers accumulated the oil on average in the amount of 2.2 ml 100 g^{-1}, which was mostly consistent with the results achieved by other authors [Hussain et al. 2011b, Stanojević et al. 2011, Najafian et al. 2012, Jianu et al. 2013]. It should be added that if a certain content of volatile oil in lavender leaves was comparable in the works of other authors [Hassanpouraghdam et al. 2011],

the flowers of lavender may be distinguished by a much higher oil concentration: 6.25–7.57% [Hassanpouraghdam et al. 2011, Danh et al. 2013, Kara and Baydar 2013,], which, most probably, results from ontogenetic and environmental variability, as well as from the manner of extraction.

The quantity of flavonoids in turn, significantly the highest in leaves (0.4%), remained on the same level in undeveloped and developed flowers (0.2%). The smallest changes in concentration concerned phenolic compounds, the amount of which was slightly bigger in flowers (0.5%) than in lavender leaves (0.4%). The examined plant material was characterized by average contents of tannins in the amount of 0.6%, which was significantly bigger (0.8%) in flower buds than in developed flowers (0.4%). Lavender leaves turned out to be an especially valuable source of flavonoids, phenolic compounds and tannins, equaling the biological value of flowers and even exceeding it (content of flavonoids). The studies of Shafaghat et al. [2012] indicated that lavender leaves contain, besides flavonoids, tannins and essential oil, also coumarins and they suggest the potential anti-microbial activity of essential oil and extracts from lavender leaves. While analyzing the chemical composition of examined lavender flowers, it was found that as they develop, the contents of essential oil and anthocyanins significantly increase, the share of tannins decreases, and the concentrations of flavonoids and phenolic compounds remains on the same level. The results of studies by Najafian et al. [2012] demonstrate the reverse pattern concerning the contents of lavender essential oil in lavender flowers before and in full blooming, the authors suggest, however, that the raw material should be harvested in the phase of full blooming due to increased share of linalool in the oil.

Table 1. Chemical constituents of lavender raw material and its antioxidant activity (AA)

Samples	Essential oil ml 100 g^{-1}	Total flavonoids %	Total phenolic acids %	Anthocyanins mg 100 g^{-1}	Tannins %	AA by DPPH Inhibition %
Leaves	0.6	0.4	0.4	3.1	0.7	77.5
Buds	2.7	0.2	0.5	4.3	0.8	85.9
Flowers	3.2	0.2	0.5	9.9	0.4	86.3
Mean	2.2	0.3	0.5	5.8	0.6	83.2
$LSD_{0.05}$	0.44	0.09	0.89	0.84	0.19	7.81

The antioxidant activity (AA) of examined material was on average 83.2% and was significantly the highest for lavender leaves (77.5%) (tab. 1). It was demonstrated that AA of lavender flower buds and flowers was comparable (respectively: 85.9 and 86.3%). High positive correlation was demonstrated between essential oil contents and antioxidant activity (R = 0.9688), total contents of phenols and AA (R = 0.9303), as well as high negative correlation between flavonoid contents and AA (R = -0.9760) (tab. 2). Antioxidant activity (AA) of plant extracts may result both from the presence of phenolic compounds, cooperation of various phytocomponents, including flavonoids [Eghdami and Sadeghi 2010, Stankevičius et al. 2010, Nuñez et al. 2012, Saeed et al. 2012, Stancheva et al. 2014], as well as the manner of extraction [Eghdami and Sadeghi

2010, Ahmed and Shakeel 2012]. Moreover, flavonoids containing hydroxyl group (s) in their structure were found more powerful antioxidants in comparison to the others [Proteggente et al. 2002]. While analyzing the chemical composition of plant extracts it is difficult to find out which of the components play the crucial role in the antioxidant system. The ability of DPPH to neutralize a free radical is frequently consistent with the high level of the total phenols and anthocyanins [Leja et al. 2007, Szwajgier et al. 2013]. On the other hand, however, the quantitative and qualitative differentiation of phenolic components have not always reflected their antioxidant abilities [Świeca et al. 2013]. Besides, the ability to form chelate rings of the metal ions and the ability to inhibit lipid peroxidation is greatest at the lowest level of condensed tannins and flavonoids, as well as at relatively constant total content of phenolic compound [Fidrianny et al. 2013]. The demonstrated high differentiation of the chemical composition of the examined leaves, flower buds and flowers resulted from ontogenetic variability and most probably was related to the complicated transformations of these compounds. What seem to be most stable, the contents of phenolic compounds in the examined organs of lavender, less dependent upon developmental factors. Interesting dependencies also result from the performed analysis of correlation coefficients between the contents of bioactive substances and antioxidant activity (AA) determined by DPPH, method, indicating the significant share of phenolic compounds and essential oil in the antioxidant potential of lavender. High negative correlation between flavonoid compounds and AA in turn, most probably results from the specific structure of these compounds, and mainly the position of OH radicles [Bunea et al. 2011]. To determine the antioxidant potential the DPPH method seems to be the most appropriate, consistent with the concentration of polyphenols [Anasini et al. 2008, Szwajgier et al. 2013]. Most scientific studies prove that AA is more correlated with the contents of phenolic compounds than with that of anthocyanins or flavonoids [Jakobek et al. 2007, Anasini et al. 2008, Li et al. 2009], which remains in consistence with the obtained results. However, there are reports about the existence of a correlation between AA measured with the use of DPPH method and total phenols and anthocyanins contents, and the coefficient of correlation between the contents of anthocyanins and AA is lower than compared with phenols contents [Saadatian et al. 2013]. Essential oils of some plants from Lamiaceae family, rich in oxygenated terpenoids mainly monoterpenes, demonstrate a significant ability to neutralize free radicals and anti-oxidant activity [Hussain et al. 2011b]. Studies on chemical composition, as well as antioxidant activity of lavender oil demonstrated a differentiated level of its activity, dependent upon the concentration and composition of examined substance [Stanojević et al. 2011, Danh et al. 2013, Hamad et al. 2013]. The antioxidant activity of examined raw material (lavender leaves and flowers) may result from high share of linalool in the oil [Hamad et al. 2013]. For the oil, besides phenolic compounds, was the main “co-author” of antioxidant potential, expressed as the ability to neutralize DPPH, and higher ability to neutralize the free radical DPPH was demonstrated for flowers containing more linalool in oil than for leaves. Besides, the process of increased accumulation of linalool in the oil in particular phases of plant development ($1.9 < 23.9 < 29.9\%$) was convergent with the increasing level of AA ($77.5 < 85.9 < 86.3\%$).

Table 2. Simple correlation coefficients between lavender compounds and antioxidant activity (AA) of raw material

Compounds	AA by DPPH Inhibition %
Anthocyanins	0.6483
Total flavonoids	-0.9760*
Total phenolic acids	0.9303*
Tannins	-0.2855
Essential oil	0.9688*

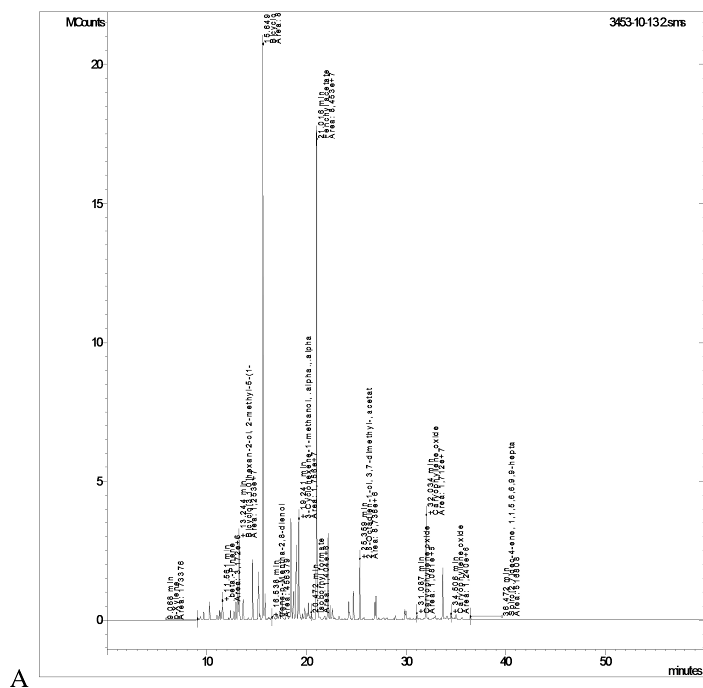
* – significant at the 0.01 level of probability

Table 3. Composition of lavender essential oil (%)

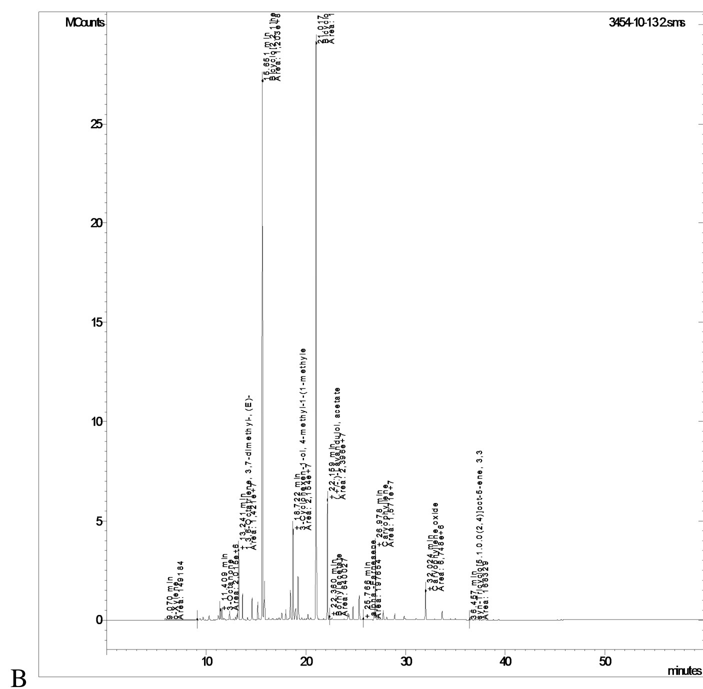
No	Compound	RI*	Leaves	Buds	Flowers
1.	Cumene	979	1.0 ±0.0	0.2 ±0.0	0.1 ±0.0
2.	*a*-Pinene	982	0.5 ±0.0	0.4 ±0.0	0.2 ±0.0
3.	Camphene	987	1.4 ±0.0	0.7 ±0.0	0.2 ±0.0
4.	Verbenene	993	0.4 ±0.0	0.2 ±0.0	Tr**
5.	Octen-3-ol	994	0.9 ±0.0	0.4 ±0.0	0.3 ±0.0
6.	3-Octanone	996	0.2 ±0.0	0.3 ±0.0	0.5 ±0.0
7.	Myrcene	997	0.1 ±0.0	0.8 ±0.0	0.6 ±0.0
8.	*dehydro*-1,8-Cineole	998	0.1 ±0.0	–	–
9.	Butyl butanoate	999	–	–	0.1 ±0.0
10.	*dehydro-cis*-Linalool oxide	1007	–	–	Tr
11.	Hexyl acetate	1012	0.4 ±0.0	0.1 ±0.0	0.5 ±0.0
12.	*p*-Cymene	1021	0.7 ±0.0	0.4 ±0.0	Tr
13.	*orto*-Cymene	1027	1.5 ±0.0	0.3 ±0.0	0.2 ±0.0
14.	Limonene	1031	3.8 ±0.2	0.7 ±0.0	0.3 ±0.0
15.	1, 8-Cineole	1035	7.3 ±0.1	1.4 ±0.0	3.4 ±0.0
16.	*γ*-Terpinene	1045	0.1 ±0.0	3.3 ±0.0	0.1 ±0.0
17.	(*E*)-*β*-Ocimene	1059	–	tr	1.2 ±0.0
18.	*trans*-Linalool oxide	1070	0.6 ±0.0	2.3 ±0.0	1.1 ±0.0
19.	*cis*-Linalool oxide	1086	0.2 ±0.0	2.1 ±0.0	0.9 ±0.0
20.	3,5-Heptadienal	1089	–	0.1 ±0.0	Tr
21.	Linalool	1097	1.9 ±0.1	23.9 ±0.2	29.9 ±0.2
22.	1-Octen-3-y-yl acetate	1101	0.2 ±0.0	1.0 ±0.0	1.7 ±0.0
23.	*trans-p*-Mentha-2,8-dien	1112	0.2 ±0.0	0.1 ±0.0	Tr
24.	Octanol acetate	1115	tr	0.1 ±0.0	Tr
25.	*trans-p*-Menth-2-en-1-ol	1123	0.2 ±0.0	0.1 ±0.0	tr
26.	*trans*-Limonene oxide	1128	0.1 ±0.0	0.1 ±0.0	0.1 ±0.0
27.	*E*-Myroxide	1136	–	0.1 ±0.2	0.1 ±0.0
28.	Camphor	1152	2.0 ±0.0	0.8 ±0.0	0.4 ±0.0
29.	4-Hexen-1-ol	1165	1.0 ±0.1	0.6 ±0.0	0.6 ±0.1
30.	Borneol	1178	11.7 ±0.3	4.3 ±0.1	1.7 ±0.1
31.	Terpinen-4-ol	1184	0.9 ±0.0	1.7 ±0.0	5.1 ±0.0
32.	Cryptone	1193	10.4 ±0.5	3.6 ±0.0	0.6 ±0.0
33.	*a*-Terpineol	1201	0.8 ±0.0	5.0 ±0.1	2.4 ±0.0

34.	*trans*-Mentha-1(7),8-dien-2-ol	1212	tr	0.2 ±0.0	0.1 ±0.0
35.	Verbenone	1219	0.7 ±0.0	0.4 ±0.0	0.1 ±0.0
36.	*trans*-Carveol	1226	0.6 ±0.0	0.2 ±0.0	Tr
37.	Nerol	1229	0.4 ±0.0	0.8 ±0.0	0.3 ±0.0
38.	Isobornyl	1238	0.7 ±0.0	0.4 ±0.0	0.1 ±0.0
39.	Linalyl acetate	1253	5.9 ±0.1	22.3 ±0.3	32.1 ±0.0
40.	Piperitone	1265	0.4 ±0.0	0.1 ±0.0	Tr
41.	Thymoquinone	1279	0.2 ±0.0	0.3 ±0.0	0.1 ±0.0
42.	Lavandulyl acetate	1289	0.7 ±0.0	3.4 ±0.1	5.9 ±0.0
43.	Bornyl acetate	1295	0.1 ±0.0	0.6 ±0.0	0.2 ±0.0
44.	*p*-Cymen-7-ol	1303	1.2 ±0.0	0.6 ±0.0	0.1 ±0.0
45.	Thymol	1308	0.4 ±0.0	Tr	Tr
46.	2,4-Cycloheptadien-1-one	1321	0.3 ±0.0	0.2 ±0.0	Tr
47.	3-*oxo*-*p*-Menth-1-en-7-al	1349	0.7 ±0.0	0.8 ±0.0	0.3 ±0.0
48.	Neryl acetate	1362	0.3 ±0.0	1.1 ±0.0	0.7 ±0.0
49.	Ni***	1380	–	2.4 ±0.1	–
50.	Sesquithujene	1421	0.1 ±0.0	Tr	Tr
51.	*a*-Santalene	1428	1.5 ±0.0	0.7 ±0.0	0.4 ±0.0
52.	*E*-Caryophyllene	1433	0.3 ±0.0	1.0 ±0.0	3.8 ±0.0
53.	*a*-*trans*-Bergamotene	1444	0.3 ±0.0	0.1 ±0.0	0.1 ±0.0
54.	*a*-26,33 Farnesene	1461	0.1 ±0.0	0.1 ±0.0	0.5 ±0.0
55.	*a*-Humulene	1474	0.1 ±0.0	0.1 ±0.0	0.2 ±0.0
56.	Germacrene D	1502	0.3 ±0.0	0.2 ±0.0	0.3 ±0.0
57.	*γ*-Cadinene	1530	3.4 ±0.1	0.4 ±0.0	0.2 ±0.0
58.	*trans*-Calamene	1537	0.3 ±0.0	0.1 ±0.0	Tr
59.	9,11-*epoxy*-Guaia-3,10(14)-diene	1576	0.7 ±0.0	0.1 ±0.0	Tr
60.	Caryophyllene oxide	1595	7.2 ±0.2	4.5 ±0.0	1.6 ±0.0
61.	Thujopsan-2-*a*-ol	1601	0.2 ±0.0	Tr	Tr
62.	Guaiol	1608	0.2 ±0.0	–	–
63.	1,10-*di*-*epi*-Cubenol	1629	1.7 ±0.0	0.2 ±0.0	Tr
64.	*epi*-*a*-Cadinol	1658	17.8 ±0.5	2.3 ±0.1	–
65.	*a*-Cadinol	1665	0.2 ±0.0	Tr	Tr
66.	*trans*-Calanen-10-ol	1673	0.8 ±0.0	0.2 ±0.0	–
67.	14-*hydroxy*-9-*epi*-(E)-Caryophyllene	1690	0.3 ±0.0	0.3 ±0.0	0.1 ±0.0
68.	*cis*-14-*nor*-Muurol-5-en-4-one	1708	2.1 ±0.1	0.3 ±0.0	–
69.	Guaia-3,10(14)-dien-11-ol	1730	0.5 ±0.0	0.1 ±0.0	–
70.	Ni	1757	1.6 ±0.1	0.1 ±0.0	tr
Total (%)			99.91	99.78	99.49

*RI – non-isothermal Kovats retention indices (from temperature-programming, using the definition of Van den Dool and Kratz [1963]) for a series of *n*-alkanes (C_6–C_{40}); **Tr – traces, contents below 0.05%; ***Ni – unidentified compound



A



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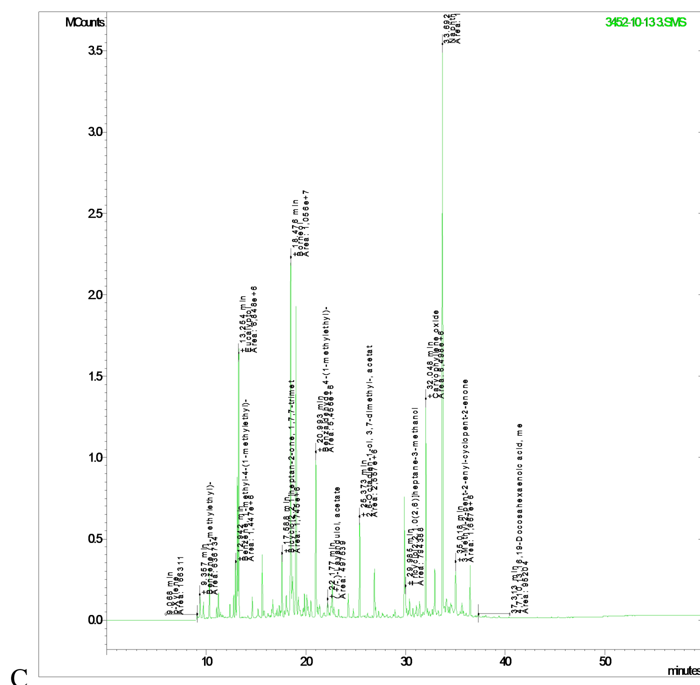

C

Fig. 1. GC-MS chromatogram of the lavender essential oil (respectively from the: leaves – A, flower buds – B and flowers – C)

The essential oil obtained from lavender flowers to a significant degree differed in its chemical composition from the essential oil distilled from lavender leaves. In the oil obtained from lavender flower buds the presence of 66 compounds was determined (tab. 3, fig. 1). The predominant compound was linalool (23.9%) and linalyl acetate (22.3%). Comparing the composition of oil obtained from flower buds to the remaining oil samples, one can notice the highest concentrations of *trans*-linalool oxide (2.3%) and *cis*-linalool oxide (2.1%), neryl acetate (1.1%), nerol (0.8%) and bornyl acetate (0.6%), similarly to *γ*-terpinene (3.3%) and terpinen-4-ol (5.0%). Analyzing the composition of essential oil obtained from developed lavender flowers the presence of 64 compounds was found, among which linalool acetate predominated (32.1%) together with linalool (29.9%). Besides, in the oil from lavender flowers the highest shares of lavandulyl acetate (5.9%), terpinen-4-ol (5.1%), *E*-caryophyllene (3.8%), 1-octen-3-y-yl acetate (1.7%) and (*E*)-*β*-ocimene (1.2%) were found, compared to the remaining ones. The chemical composition of LEO undergoes different kinds of variability: ontogenetic [Hassanpouraghdam et al. 2011, Najfian et al. 2012], genetic and environmental [Hamad et al. 2013, Jianu et al. 2013]. Besides, high variability of EOL chemical composition and of its bioactivity results from different extraction methods [Jakobek et al. 2007, Stanojević

et al. 2011, Danh et al. 2013, Stancheva et al. 2014]. The obtained shares of 1,8-cineole, caryophyllene oxide and linalool acetate were partly refers to the results achieved by other authors [Hassanpouraghdam et al. 2011, Shafaghat et al. 2012]. Linalyl acetate and linalool belong to the most frequently determined tents of these components are especially important due to their antiviral activity [Orhan et al. 2012], as well as the anti-microbial and antioxidant activity of oils rich in these components [Danh et al. 2013]. The studies by Jianu et al. [2013] suggest that anti-microbial activity of lavender oils results from antibacterial properties of main and secondary components of the oil. In the group of main LEO components there are also: borneol [Afsharypuor and Azarbayejany 2006], 1,8-cineole [Jianu et al. 2013], menthol [Rostami et al. 2012], camphor [Kara and Baydar 2013], β-phelandrene and caryophyllene [Jianu et al. 2013]. The share of above-mentioned components most probably results from environmental changeability, though it can also be related to the term of raw material harvest and the manner of oil distillation. From among the components of the examined lavender oil occurring in larger quantities and/or with significant biological activities, attention should be paid to the increased share of linalool (1.9 < 23.9 < 9.9%), linalyl acetate (5.9 < 22.3 < 32.1%), lavandulyl acetate (0.7 < 3.4 < 5.9%), *E*-caryophyllene (0.3 < 1.0 < 3.8%) and the decreasing contents of limonene (3.8 > 0.7 > 0.3%), camphor (2.0 > 0.8 > 0.4%), borneol (11.7 > 4.3 > 1.7%), thymol (0.4% > tr > tr), caryophyllene oxide (7.2 > 4.5 > 1.6%), *epi*-α-cadinol (17.8 > 2.3 > 0%) in the oil, as plant development (leaves – flower buds – flowers). Similarly Najafian et al. [2012] found higher share of linalool in the oil and lower share of borneol and caryophyllene oxide in full blooming period than before lavender blooming period. One of the components of examined lavender oil was 1,8-cineole, a compound with anti-microbial and anti-cancer potential [Hendry et al. 2009, Wang et al. 2012], acting as a protection for blood vessels [Lahlou et al. 2002]. The concentration of 1,8-cineole in the examined lavender oil was the highest in the samples distilled from leaves (7.3%), then it was decreasing in the oil from flower buds (1.4%), and next it increased in the further stage of flowers development (3.4%). Other dependencies were demonstrated by Najafian et al. [2012], examining lavender oil in Iran, determining the highest share of 1,8-cineole before blooming. It should be noticed that the oil distilled from lavender leaves from Iraq was characterized by significantly higher share of 1,8-cineole: 17.6–31.9% [Hassanpouraghdam et al. 2011, Shafaghat et al. 2012], which can indicate the effect of temperature during plant growth period upon the accumulation of that component. Besides, the quantitative and qualitative changes in certain components of lavender oil, demonstrated in the foregoing studies and in those of other authors, most probably result from transformations of particular compounds produced in separate metabolic routes. The composition of monoterpenes and level of aliphatic alcohols seem to be more dependent upon the phenological phase of the cycle than on sessquiterpenes [Schwob et al. 2004].

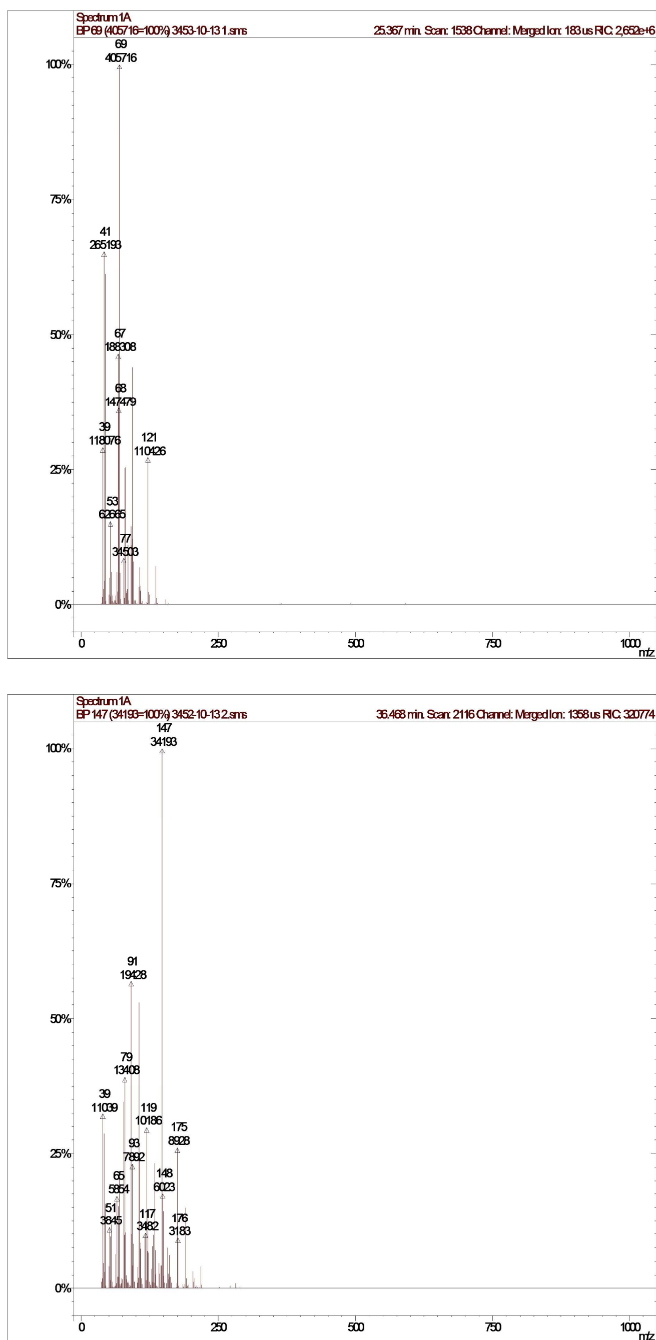

Fig. 2. Spectrum of unidentified compounds (respectively from the left: RI 1380 and RI 1757)

CONCLUSIONS

The demonstrated rich chemical composition of leaves, flower buds and flowers of medicinal lavender, as well as their antioxidant activity, indicate the possibility of broader application of these raw materials in pharmaceutical, cosmetic and food industry. Positive high correlation between the essential oil and phenolic acids contents and the ability to reduce DPPH indicate that the volatile oil and phenolic compounds are main co-authors of the antioxidant activity of medicinal lavender leaves and flowers. The results of the foregoing studies also suggest that the antioxidant activity of lavender (77.5–86.3%) is more correlated with the contents of essential oil and phenolic compounds than antocyanins, flavonoids and tannins. Lavender flowers, regardless of the phase of their development had greater concentrations of essential oil and linalool, as well as linalyl acetate in the oil, as well as phenolic compounds and antocyanins, together with greater antioxidant potential compared to leaves, which were, in turn, richer in flavonoids and tannins (only regarding fully developed flowers). Lavender oil has rich chemical composition, which is quantitatively and qualitatively variable in the plant development process. The predominant components in the oil distilled from leaves were: *epi*-*α*-cadinol (17.8%), cryptone (10.4%), 1,8-cineole (7.3%) and caryophyllene oxide (7.2%). The oil obtained from lavender flowers was, in turn, characterized by high share of linalyl acetate (22.3–32.1%) and linalool (23.9–29.9%).

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SKŁAD CHEMICZNY I AKTYWNOŚĆ ANTYOKSYDACYJNA CZĘŚCI NADZIEMNYCH LAWENDY (*Lavandula angustifolia* Mill.)

Abstract. Badania biochemiczne obejmowały liście, pąki kwiatowe i kwiaty lawendy. Wykazano pozytywną korelację pomiędzy zawartością olejku eterycznego i aktywnością antyoksydacyjną (AA) (R = 0.9688), kwasów fenolowych i AA (R = 0.9303),wysoką oraz negatywną korelację pomiędzy zawartością flawonoidów a AA (R = -0.9760). Wyniki badań wskazują ponadto, że AA lawendy (77.5–86.3%) jest bardziej skorelowana z zawartością olejku eterycznego i kwasów fenolowych, niż flawonoidów, antocyjanów i garbników. Głównymi składnikami olejku otrzymanego z liści był *epi*-*α*-kadinol (17.8%), krypton (10.4%), 1,8-cyneol (7.3%) i tlenek kariofilenu (7.2%), natomiast w olejku destylowanym z kwiatów dominował octan linalylu (22.3–32.1%) i linalol (23.9–29.9%).

Słowa kluczowe: kwasy fenolowe ogółem, aktywność redukcji rodnika DPPH składniki olejku eterycznego

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