

Antioxidant capacity and total polyphenol content of *Lavandula* cultivars at different growing areas in Hungary

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Summary: *Lavandula* species are worldwide grown essential oil producing medicinal plants with considerable economic value. Beside volatile oil, lavender also contains different phenolic compounds which have been less widely studied till now. Cultivation of lavender has become widespread in Hungary in the recent years, however, growers have limited knowledge on the productivity of cultivars available. In our research we were aimed to studying the antioxidant capacity and total polyphenol content of samples collected in two growing areas (Dörgicse and Szomód) from flowers of two *L. angustifolia* ('Hidcote', 'Munstead') and two *L. × intermedia* ('Grosso', 'Grappenhall') varieties, during the full blooming period of 2017. Antioxidant capacity of the samples was determined by FRAP assay (Benzie and Strain, 1996), while total polyphenol content (TPC) was measured by a modified method of Singleton and Rossi (1965). According to our results, varieties of *L. × intermedia* showed higher values of antioxidant capacity and of total polyphenol content, than those of *L. angustifolia* cultivars. Among them, both the antioxidant capacity (179.6 mg AAE/g DW) and total polyphenol content values (152.4 mg GAE/g DW) of 'Grosso' from Dörgicse were the highest. Concerning FRAP values of all the cultivars investigated, larger variability were found in Dörgicse, than in Szomód. Regarding the effect of growing area, each cultivar represented similar FRAP values in Dörgicse and in Szomód, except for 'Grosso'. However, in the case of TPC values, higher variability was observed between the growing areas, especially in the case of 'Grosso'. In the future our studies on lavender polyphenols will be completed with qualitative evaluation of the values obtained by HPLC analysis.

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Key words: lavender cultivars, antioxidant capacity, total polyphenol content, growing area

Abbreviations

AAE ascorbic acid equivalent
DW dry weight
FRAP ferric reducing antioxidant power

GAE gallic acid equivalent
TPC total polyphenol content

Introduction

Lavender is a valuable essential oil producing medicinal plant belonging to the *Lamiaceae* family. Several species are involved in the genus *Lavandula*, among them, *L. angustifolia* and *L. x intermedia* are the most important. Both species are mainly grown for their essential oil, which is mainly used in the cosmetic industry and perfumery, in addition in aromatherapy (Lis-Balchin, 2002; Grant et al., 2011). However, it is important to draw the attention to other biologically active compounds accumulated in the plant, including polyphenols. Aqueous or methanolic extracts from lavender flowers or leaves also show beneficial pharmacological effects owing to phenoloid compounds. The most important property associated with polyphenols is the antioxidant activity. Natural antioxidants are applied in health care as well as in food preservation. The safety of the synthetic antioxidants have been questioned. Several preservatives have a destructive effect on human skin and they can be a possible carcinogenic agent (Darbre et al., 2004). The majority of preservatives of the cosmetics industry are artificially synthesized chemical compounds. However, currently the

attention of many researchers has been focused on the usage of medicinal plants as sources of natural antioxidants.

According to Ceylan et al. (2015) methanolic extract of *Lavandula stoechas* might be a new potential natural antioxidant source, due to high antioxidant activity. Torras-Claveria et al. (2007) and Sánchez-Vioque et al. (2013) also proved antioxidant activity and total polyphenol content of lavandin waste after essential oil distillation with different extraction methods. In the study of Andrys et al. (2017), *L. angustifolia* 'Munstead' was evaluated, however, the value of 9.21 ± 0.64 mg TE g⁻¹ DW by FRAP method, was significantly lower than the other two cultivars involved ('Ellagance Purple', 'Blue River'). Based on the study of Nurzyńska-Wierdak & Zawislak (2016), high positive correlation was found between the essential oil contents and antioxidant activity (AA) (R = 0.9688), and total phenolic acids (AA) (R = 0.9303) at *L. angustifolia* samples. It was also established by Shafaghat et al. (2012); and Zielinska & Matkowski (2014) that the synthesis and accumulation of other polyphenols is changing during plant development and depend upon climatic factors. The antioxidant activity of plant extracts

can be explained by the structure of phenolic compounds. Considering the above mentioned, the essential oil and phenolic content as well as their corresponding antioxidant potential of diverse plant organs were assayed by Nurzyńska-Wierdak & Zawiślak (2016). According to their study antioxidant activity (AA) of leaves, flower buds and flowers were 77.5%, 85.9% and 86.3%, respectively. In the research of Blažeković (2010), authors evaluated the effectiveness of inflorescence stalk extracts, as well as flower and leaf. It was shown that the leaf extracts of the two *Lavandula* taxa involved were the most active, followed by flower extracts, while the inflorescence stalk extracts showed lower antioxidant activity. In the study of Duda et al. (2015), higher amount of phenolics are detected at the beginning of flowering than in full bloom phase.

According to previous studies, a lack of scientific information can be seen on evaluating the effect of the environmental conditions on the polyphenol production of *Lavandula* varieties, while it is also an important issue. In our study, it was the first time that we have investigated the antioxidant capacity and total polyphenol values of different cultivars of *L. angustifolia* and *L. × intermedia* grown in distinct areas of Hungary. Our aim was to evaluate the effects of genotype as well as environmental factors on the FRAP and TPC values of *Lavandula* samples collected from 2 lavender and 2 lavandin cultivars in full flowering phase from 2 growing areas (Dörgicse, Szomód).

Materials and methods

Plant material and sampling

Two *L. angustifolia* (true lavender) cultivars, 'Munstead' (Mu) and 'Hidcote' (Hi), as well as two *L. × intermedia* (lavandin) varieties, 'Grosso' (Gro), and 'Grappenhall' (Gra) were studied. Sampling was carried out on 14th June (true lavender), and 14th July (lavandin), at full blooming period, in 2017 in 3 replications at each variety. All the flower samples were dried and stored at room temperature until the laboratory analysis.

Growing areas

Growing areas are located in the Northern Transdanubia: in Dörgicse and Szomód where the lavender fields were established in 2003, and 2013, respectively. Dörgicse lies on 200 m high on Balaton Uplands, and has rocky brown forest soil with limestone base rock. There the climate is moderately warm, and the average annual rainfall is higher than 600 mm. Szomód is a small village near Tata. It has sandy brown forest soil with loess base rock. The climate of Szomód is colder than the average climate at other areas of the country, and it has average annual rainfall between 650-700 mm. General environmental conditions and soil characteristics of the distinct growing areas are included in **Table 1/a** and in **Table 1/b**, while monthly precipitation values prior to harvest are also shown in **Table 1/c**.

Preparation of extracts

Dried flower samples were turned into powder by grinding. 0.5 g of the powder was extracted by 50 ml boiling distilled water and was kept at room temperature for 24 hours. Then the extracts were filtered and stored in freezer prior the measurements.

Table 1/a. Environmental conditions of the growing areas involved.

Study conditions	Characteristics of the growth areas	
	Dörgicse	Szomód
Region	Balaton Uplands, Veszprém County	NE-Transdanubia, Komárom Esztergom County
Exposure of growing area	plain	NW
Location	46° 55' 01" N, 17° 43' 19" E	47° 40' 57" N, 18° 20' 30" E
Base rock	limestone	loess
Soil type	rocky brown forest soil	sandy brown forest soil
Average annual temperature, °C	9-10	8-9
Average annual rainfall, mm	600-650	650-700
Lavender field established in	2003	2013

Ferric reducing/antioxidant power (FRAP) assay

Ferric reducing/antioxidant power of *Lavandula* extracts was measured by the FRAP assay, according to the slight modification of the method of Benzie and Strain (1996). Reagents for the method were obtained from Sigma-Aldrich: Sodium-acetate, acetic acid, TPZ (2,4,6-tripiridil-s-triazine, hydrogen-chloride, iron-chloride. FRAP reagent was prepared prior to the measurements to contain sodium acetate buffer (pH=3.6), TPZ (2,4,6-tripiridil-s-triazine) in HCL and FeCl₃*6H₂O solution (20 mmol/l), in proportion of 10:1:1 (v/v/v), respectively. 10-20-30 µl of test sample was added to 1.5 ml of acting FRAP reagent and 40-30-20 µl distilled water then the absorbance was recorded at λ=593 nm after 5 min using the above spectrophotometer. A blank of distilled water was applied. FRAP values of samples were calculated from a standard curve equation and expressed in ascorbic acid equivalent based on the dry extract weight and expressed in mg AAE/g DW. The measurements were done in 3 replications in the case of every sample.

Total polyphenol content assay

Total phenol content of the extracts was determined by method of Singleton and Rossi (1965) with slight modifications. Reagents for the assay were purchased from Sigma-Aldrich: methanol, Folin-Ciocalteu reagent, sodium-carbonate, gallic acid. 25-50 µL of sample solution was placed into a test tube then mixed with 2.5 mL Folin–Ciocalteu's reagent (10 v/v%). After 1 min of incubation, 2 ml of sodium carbonate (0.7 M) was also added. The absorbance of the solution was measured at the wavelength of λ=760 nm in a Thermo Evolution 201 spectrophotometer after a 5 min incubation period in hot water (50 °C) in comparison with the blank of distilled water. Gallic acid (0.3 M) was used as a chemical standard for calibration. The total phenolic content of the samples was expressed in gallic acid equivalent calculated on the dry weight basis of the extract (mg GAE/g DW). The measurements were done in 3 replications.

Table 1/b. Soil characteristics of the growing areas of *Lavandula* in Dörgicse and Szomód (According to soil analyses of the growers).

Growing locations	Soil parameters												
	pH	K _A	CaCO ₃ m/m %	Humus m/m %	NO ₃ ⁻ +NO ₂ ⁻ -N mg/kg	P ₂ O ₅ mg/kg	K ₂ O mg/kg	Mg mg/kg	Na mg/kg	Zn mg/kg	Cu mg/kg	Mn mg/kg	SO ₄ ²⁻ mg/kg
Dörgicse	7	43	5	2	11	261	117	317	116	14	2	214	22
Szomód	8	37	11	2	13	303	263	69	38	1	0	26	53

Table 1/c. Quantity of precipitation (mm) prior to harvest period in 2017 (mm/month) (data were provided by OMSZ-National Meteorology Service).

Growing area	Amount of precipitation (mm/month)	
	Dörgicse	Szomód
January	20.3	23.0
February	46.5	20.8
March	20.9	41.3
April	53.8	70.2
May	15.2	23.6
June	81.6	21.1
Total	238.3	200.0

Table 2. Evaluation of the effect of cultivars on the antioxidant capacity (FRAP) and total polyphenol content (TPC) in both growing areas (Dörgicse and Szomód) involved.

Growing area	Species	Cultivars	Effect of cultivars	
			FRAP	TPC
Dörgicse	<i>L. angustifolia</i>	'Hidcote'- 'Munstead'	p<0.0050**	p<0.0020**
		'Grosso'- 'Grappenhall'	p<0.0001**	p<0.0020**
	All cultivars	p<0.0001**	p<0.0001**	
Szomód	<i>L. angustifolia</i>	'Hidcote'- 'Munstead'	p<0.1510	p<0.0260*
		'Grosso'- 'Grappenhall'	p<0.2510	p<0.0460*
	All cultivars	p<0.0001**	p<0.0001**	

Legends: *: significant difference at p<0.05; **: significant difference at p<0.01

Statistical analysis

The antioxidant activity as FRAP (mg AAE/g DW) and total polyphenol content as TPC (mg GAE/g DW) values were shown as means±standard deviations. Datas were analyzed by the IBM SPSS Statistics 23, using one-way ANOVA to compare the FRAP and TPC values of each cultivar, where the significance level was set at p<0.05. Homogeneity of variances was checked by Levene's test.

Results

Effect of genotype

Dörgicse

According to our study in Dörgicse, significant differences were observed among all the *Lavandula* cultivars investigated concerning values of FRAP (p<0.0001) and TPC (p<0.0001) (Table 2). Among *L. angustifolia* varieties ('Hidcote' and 'Munstead') significant differences were found regarding values of FRAP (p<0.005) and TPC (p<0.002). *L. × intermedia* cultivars ('Grosso' and 'Grappenhall') also differed significantly at FRAP (p<0.0001) and TPC (p<0.002) values. The lowest FRAP content was possessed by 'Munstead' (104.9 mg AAE/g DW), while the highest value was represented by 'Grosso' (179.6 mg AAE/g DW) (Figure 1). That tendency was observed at TPC values either: lowest value was detected at

'Munstead' (113.3 mg GAE/g DW), and the highest value at 'Grosso' (152.4 mg GAE/g DW). In this respect, correlation was found between antioxidant activity and total polyphenol content values of the cultivars.

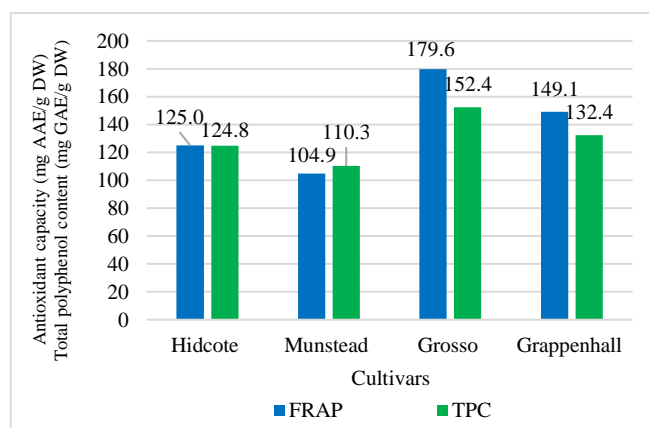
Szomód

In Szomód FRAP and TPC values of all the cultivars involved differed significantly (p<0.0001) (Table 2.). However, FRAP values did not changed considerably neither between *L. angustifolia* varieties ('Hidcote' and 'Munstead') (p<0.151), nor between *L. × intermedia* cultivars ('Grosso' and 'Grappenhall') (p<0.251) did not differed significantly. Besides, significant differences were observed concerning TPC values of 'Hidcote' and 'Munstead' (p<0.026) and in the case of 'Grosso' and 'Grappenhall' (p<0.046) (Table 2).

It was also found that the antioxidant capacity (116.8 mg AAE/g DW) as well as TPC (93.4 mg GAE/g DW) values were the lowest at 'Munstead' among all the cultivars investigated (Figure 2). However, the highest values of FRAP (150.3 mg AAE/g DW) and TPC (140.8 mg GAE/g DW) were detected at 'Grappenhall' (Figure 2).

Effect of growing areas

Regarding antioxidant capacity (FRAP), the effect of growing area was significant only at 'Grosso' (p<0.0001). However, in the case of total polyphenol content (TPC) values, significant differences were found at all the investigated varieties (Table 3). The total polyphenol content values at *Lavandula* cultivars were higher in Dörgicse than in Szomód, except for 'Grappenhall' (Figure 4). According to soil analyses, higher amounts of CaCO₃ and macronutrients (NPK) were measured in Szomód, while the soil of Dörgicse was richer in meso- and microelements (Table 1/b). The differences in precipitation prior to harvest between the growing areas was also considerable (38.3 mm) (Table 1/c). These ecological features might have effect on the polyphenol biosynthesis of *Lavandula* varieties involved.

**Figure 1.** Antioxidant capacity (FRAP: mg AAE/g DW) and total polyphenol content (TPC: mg GAE/g DW) values of *L. angustifolia* and *L. × intermedia* cultivars in Dörgicse (2017).

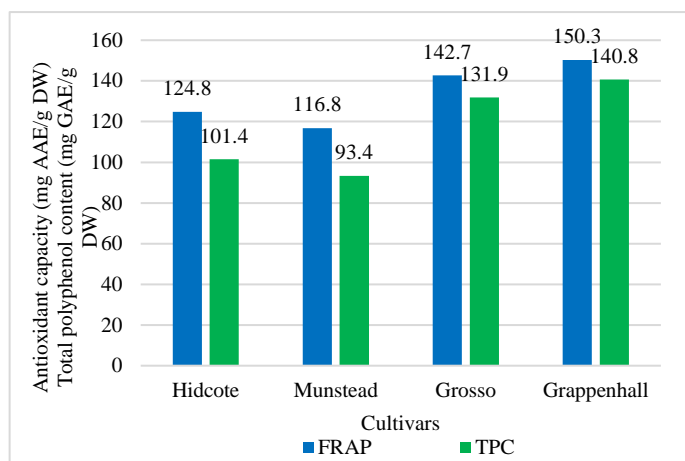


Figure 2. Antioxidant capacity (FRAP: mg AAE/g DW) and total polyphenol content (TPC: mg GAE/g DW) values of *L. angustifolia* and *L. x intermedia* cultivars in Szomód (2017).

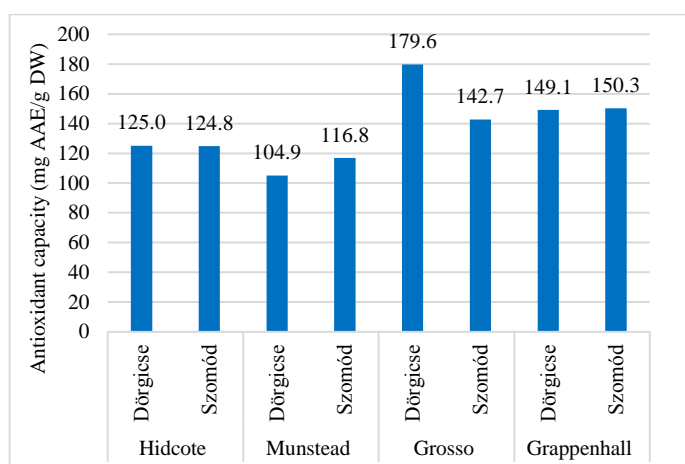


Figure 3. Antioxidant capacity (FRAP: mg AAE/g DW) of *L. angustifolia* and *L. x intermedia* cultivars in Dörgicse and Szomód (2017).

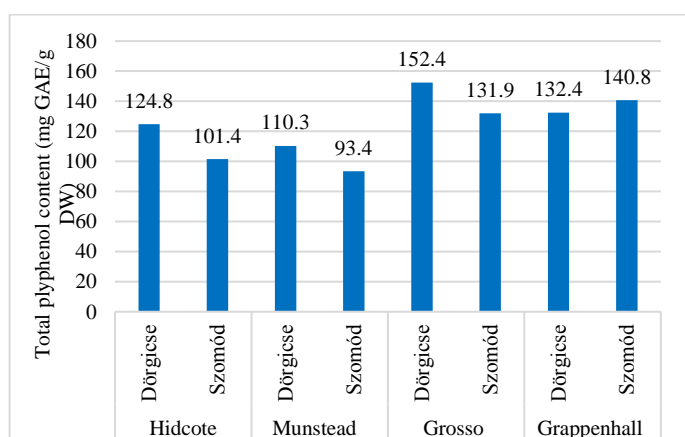


Figure 4. Total polyphenol content (TPC: mg GAE/g DW) of *L. angustifolia* and *L. x intermedia* cultivars in Dörgicse and Szomód (2017).

Discussion

According to our results, it can be concluded that statistically proven differences were found concerning antioxidant capacity values (FRAP), influenced by the genotype of *Lavandula* cultivars, regardless of the growing areas. Besides, it was observed, that the variability of antioxidant capacity values was higher in Dörgicse than in Szomód. Moreover, only FRAP values of 'Grosso' were significantly affected by the growing area

($p < 0.0001$) (Table 3). On the contrary, total polyphenol content values of lavender samples differed significantly not only by the genotype (Table 2) but also by the growing area (Table 3). Therefore, it was important to summarize the main characteristics of the cultivars, as a conclusion of this study.

Table 3. Evaluation of the effect of growing area on the antioxidant capacity (FRAP) and total polyphenol content (TPC) of cultivars involved.

Species	Cultivars	Effect of growing area	
		FRAP	TPC
<i>L. angustifolia</i>	'Hidcote'	$p < 0.6280$	$p < 0.0001^{**}$
	'Munstead'	$p < 0.0620$	$p < 0.0001^{**}$
<i>L. x intermedia</i>	'Grosso'	$p < 0.0001^{**}$	$p < 0.0020^{**}$
	'Grappenhall'	$p < 0.8000$	$p < 0.0010^{**}$

Legends: *: significant difference at $p < 0.05$; **: significant difference at $p < 0.01$

Table 4. Correlation between the antioxidant capacity (FRAP) and total polyphenol content (TPC) values of cultivars involved in different growing areas.

Species	Cultivars	Growing area	
		Dörgicse	Szomód
<i>L. angustifolia</i>	'Hidcote'	$p < 0.9700$	$p < 0.0001^{**}$
	'Munstead'	$p < 0.2090$	$p < 0.0001^{**}$
<i>L. x intermedia</i>	'Grosso'	$p < 0.0001^{**}$	$p < 0.1110$
	'Grappenhall'	$p < 0.0001^{**}$	$p < 0.0320^*$

Legends: *: significant difference at $p < 0.05$; **: significant difference at $p < 0.01$

'Hidcote'

Among *L. angustifolia* cultivars, 'Hidcote' possessed the highest values of antioxidant activity (125.0 mg AAE/g DW) and total polyphenol content (124.8 mg GAE/g DW) in both growing areas (Figure 3-4). The values of FRAP and TPC in Dörgicse were almost the same (125.0 mg AAE/g DW and 124.8 mg GAE/g DW) ($p < 0.970$), however, in Szomód larger variability could be observed in these values ($p < 0.0001$) (Table 4), which could be due to the lower TPC values (Figure 2).

'Munstead'

This variety represented the lowest values of antioxidant activity (104.6 mg AAE/g DW) and total polyphenol content (93.4 mg GAE/g DW) in both growing areas (Figure 3-4). Values of FRAP and TPC in Dörgicse did not differ significantly ($p < 0.209$), however, as in the case of 'Hidcote', in Szomód these values showed significant differences ($p < 0.0001$) (Table 4).

'Grosso'

The FRAP and TPC values were the highest at this variety among the cultivars involved (FRAP: 179.6 mg AAE/g DW; TPC: 152.4 mg GAE/g DW, both in Dörgicse). At 'Grosso', antioxidant capacity and total polyphenol content values were significantly influenced by the effect of growing area (FRAP: $p < 0.0001$, and TPC: $p < 0.002$) (Table 3). According to statistical analyses between FRAP and TPC values of 'Grosso' significant differences were observed in Dörgicse ($p < 0.0001$), while these values did not differ significantly in Szomód ($p < 0.111$) (Table 4).

'Grappenhall'

In Szomód this variety represented the highest values of FRAP and TPC among *Lavandula* varieties, respectively (FRAP: 150.3 mg AAE/g DW, and TPC: 140.8 mg GAE/g DW) (Figure 2). According to statistical analyses between FRAP and TPC values of 'Grappenhall' in Dörgicse significant differences were observed ($p < 0.0001$), moreover these values also differed significantly in Szomód ($p < 0.032$) (Table 4).

Evaluating the investigated cultivars, we concluded, that the two *L. × intermedia* cultivars, ‘Grosso’ and ‘Grappenhall’ may be candidates for Hungarian growers in order to contribute higher antioxidant capacity values of the products created. Antioxidant activity values of ‘Grosso’ were more variable by the growing areas, besides in this respect ‘Grappenhall’ was a more stable cultivar.

According to our knowledge, *Lavandula* species prefers dry and calcareous soil (Bernáth et al., 2013). In the soil of Dörgicse less CaCO₃ values (m/m %) and higher microelement amounts were detected, which also could induce accelerated secondary metabolite production (polyphenols). This might be an explanation to the high FRAP and TPC values of ‘Grosso’ and higher TPC values of the other *Lavandula* cultivars from Dörgicse. However, our findings do not correspond to those reported by previous authors (Trócsányi et al., 2015; Németh-Zámbori et al., 2016; Radácsi et al., 2016) concerning drought stress reactions of other xerophil species, such as *Thymus vulgaris*. According to their results, it was concluded that more polyphenols, such as rosmarinic acid were detected in thyme and lemon balm plants as a consequence of water deficiency, while in our experiment this phenomenon was not observed in the growth year of 2017. *Lavandula* cultivars, especially ‘Grosso’, showed higher total polyphenol content values in the growing area of Dörgicse, where more precipitation was detected. However, further investigations are needed to confirm our findings concerning factors influencing polyphenol production of *Lavandula* cultivars among different growing conditions, completed by HPLC analysis of phenolic compounds.

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