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## ANTIOXIDANT CAPACITY AND COMPOSITION OF ESSENTIAL OIL OF LAVENDER AND LAVANDIN GROWING IN BOSNIA AND HERZEGOVINA

### SUMMARY

The *Lavandula* genus is one of the world's most popular medicinal and aromatic plants and is rich in essential oil. Lavender (*Lavandula angustifolia* Mill.) and lavandin (*Lavandula intermedia* Emeric ex Loisel.) have high commercial values worldwide. However, their quality depends on genetic properties, environmental conditions, and cultural practices. Therefore, this research aimed to determine the antioxidant capacity and content of constituents in the essential oil of lavender and lavandin grown in the environmental conditions of central Bosnia and Herzegovina (B&H). Samples of lavender and lavandin (cv. grosso) were collected at Butmir, B&H. The tested properties were total phenolic, total flavonoids, antioxidant activity, essential oil content and content of constituents in essential oil. The quality of tested plants was statistically significantly dependent on the cultivar. The highest values of total phenolic, flavonoids, and antioxidant capacity were recorded in the lavender (54.26 mg GAE g<sup>-1</sup>, 41.49 mg CAE g<sup>-1</sup> and 17.49 μM Fe<sup>2+</sup> g<sup>-1</sup>, respectively), while the lowest was in the lavandin (39.30 mg GAE g<sup>-1</sup>, 24.07 mg CAE g<sup>-1</sup> and 10.84 μM Fe<sup>2+</sup> g<sup>-1</sup>, respectively). Essential oil content ranged from 4.44 (Lavender) to 8.25 mL 100 g<sup>-1</sup> (lavandin). Essential oil of lavender and lavandin were rich in linalool.

**Keywords:** lavender, lavandin, antioxidant capacity, essential oil, GC-MS

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## INTRODUCTION

Since ancient times, people have been fascinated with medicinal and herbal plants because of their beneficial effects on human health. Lavender is one of the world's most popular medicinal and aromatic plants. Although there are 39 different species in this genus *Lavandula*, only three species have high commercial values, including Lavender (*Lavandula angustifolia* Mill.), lavandin (*Lavandula intermedia* Emeric ex Loisel.) and spike lavender (*Lavandula latifolia* Medik.) (Sönmez *et al.*, 2018). Therefore, the cultivation of these species as therapeutic and aromatic herbs has increased significantly over the past few years (Kıvrak, 2018). Currently, the most famous countries for cultivating Lavender are Bulgaria, France, Italy, Spain, Turkey, Croatia, Greece, Kashmir, South Africa, and regions in Northern Africa (Vijulie *et al.*, 2022).

Lavender is primarily cultivated for its essential oil, which has multiple uses. Lavender essential oil is highly valued in cosmetics, food preservation, agroindustry, traditional medicine, etc (Białoń *et al.*, 2019; Mambri *et al.*, 2018; Wells *et al.*, 2018; Komnenić *et al.*, 2020). Lavender is used in traditional medicine for the treatment of various diseases (Behmanesh *et al.*, 2015; Cardia *et al.*, 2018; Firoozeei *et al.*, 2021; Koulivand *et al.*, 2013; Lis-Balchin *et al.*, 1999; Pandur *et al.*, 2021; Puškárová *et al.*, 2017). The medicinal properties of all medicinal plants, including lavender, depending on the content of phenolic compounds and their antioxidant capacity (Gavrić *et al.*, 2023). Although genetic processes control the amount of these components in the plant, environmental factors and cultivation method also significantly impact their contents (Gavrić *et al.*, 2018a; Albergaria *et al.*, 2020; Mohammadi *et al.*, 2021; Lebedev *et al.*, 2022). However, it is known that all cultivars accumulate phenolic compounds, which are characterized by high antioxidant activity (Wells *et al.*, 2018).

The quality of lavender strongly depends on the content of the essential oil and its composition of constituents. Lavender oil can have more than 100 components, most of which are terpene compounds (Sałata *et al.*, 2020). Previous research (Białoń *et al.*, 2019; Mambri *et al.*, 2018; Minji Hong, 2022; Sałata *et al.*, 2020) has shown that lavender and lavandin essential oil contains: linalyl acetate, linalool, and  $\gamma$ -cadinene, p-cymen-8-ol, borneol, lavandulol, o-cymene, bornyl acetate, (E)-caryophyllene, eucalyptol, camphor. However, according to many researchers (Białoń *et al.*, 2019; Erhatic *et al.*, 2020; Kvaternjak *et al.*, 2020; Wells *et al.*, 2018), the concentration of these components in essential oils varies greatly depending on cultivars and environmental factors. For example, lavender essential oil contains 17.8-50.0% linalool, 10.1-54.0% linalyl acetate, and traces of camphor, while lavandin essential oil contains 22.5-35.5% linalool, 23.6-35.4% linalyl acetate and in 6.3-12.2% camphor (Lesage-Meessen *et al.*, 2015). On the other hand, it was found that the essential oil composition depends on the growing area's weather conditions. For example, the content of linalool, the main compound of lavender oil, increases with the increase in average daily temperature, while its content is reduced during the rainy season (Hassiotis *et al.*, 2014).

Many studies in the literature study the composition of essential oils and the antioxidant capacity of lavender, but there are no studies in Bosnia and Herzegovina (B&H). Therefore, this research aimed to determine the antioxidant capacity and content of essential oil constituents of lavender and lavandin grown in the environmental conditions of central Bosnia and Herzegovina (B&H).

## MATERIAL AND METHODS

### Plant material and sampling

Lavender (*Lavandula angustifolia*) and lavandin (*Lavandula intermedia* cv. grosso) were used in this study. Samples were collected at Butmir, B&H (Experimental field of Sarajevo Faculty of Agriculture and Food Sciences, 43°49'34.42" N, 18°19'18.48" E; 505 m a.s.l.).

### Materials for analysis

The total phenol content, total flavonoid content, antioxidant activity, and essential oil contents is determined in inflorescence samples. Samples were collected at the full flowering stage (July 19, 2021). The samples were dried in a dim environment for 30 days at room temperature. After that, collecting samples were dried and then ground to a size of 1 mm.

### Extract preparation

Briefly, 1 g of each ground sample was added to a 100 mL volumetric flask, and the flask volume was filled with 60% ethanol and mixed. Then the extracts were filtered and stored in the refrigerator before the measurements.

### Determination of total phenols content

The total phenolic contents of ethanolic extracts of lavender and lavandin were estimated using the Folin Ciocalteu reagent as described by Bystrická *et al.* (2011). Results were expressed as mg gallic acid equivalents per gram (mg GAE g<sup>-1</sup>).

### Determination of total flavonoid content

The flavonoid content of lavender and lavandin ethanolic extracts was determined using spectrophotometric methods based on forming aluminum-flavonoid complexes (Gavrić *et al.*, 2018b). Results were expressed as mg catechin per gram (mg CAE g<sup>-1</sup>).

### Determination of antioxidant activity

The total antioxidant capacity of the extract was measured using the FRAP (ferric reducing antioxidant power) method described by Benzie and Strain (1996). Results was expressed as  $\mu\text{M Fe}^{2+} \text{ g}^{-1}$  of the dry sample.

### Extraction and determination content of essential oils

Extracting essential oils from flower samples was carried out according to the protocol previously described by Clewenger (1928). Briefly, the dried flower samples were weighed (50 g) and then transferred to a 1000 mL volumetric flask with 500 mL of distilled water. The samples were distilled for two hours. The volume of essential oil was measured after distillation. After that, the essential oils were dried with sodium sulfate anhydrous, packed in dark vials, and stored in the refrigerator until analysis.

### Gas Chromatography/Mass Spectrometry Analysis

The compositions of extracts were quantified by means of gas chromatograph with autosampler (Agilent Technologies, 7820A) equipped with capillary column (Agilent HP5-ms ultra-inert, 30 m×320 μm ×0.25 μm) coupled to an Agilent Technologies Mass Selective Detector (MSD-5977E). Temperature program used: 60 °C (1 min hold) to 246 °C (0 min hold) at a rate of 3 °C/min, and then to 280 °C at a rate of 10 °C/min. Injection volume of 1 μL was used in splitless injection mode at 220 °C and pressure of 8.4599 psi with carrier gas He of 99.9995 % purity.

### Statistical methods

All experiments were performed in triplicate. One-way ANOVA was used to examine the data at a significance level of 0.05. The research findings were statistically analyzed using the SPSS 22 software.

## RESULTS AND DISCUSSION

In this research, the amounts of total phenolic, flavonoids, and antioxidant capacity of the extracts from the inflorescence are shown in table 1.

Table 1. Effect of cultivars on total phenolic contents, total flavonoid contents, antioxidant capacity, and essential oil content

Cultivar	Total phenolics	Total flavonoids	Antioxidant capacity	Essential oil contents
	mg GAE g <sup>-1</sup>	mg CAE g <sup>-1</sup>	μM Fe <sup>2+</sup> g <sup>-1</sup>	mL 100 g <sup>-1</sup>
Lavander	54.26a	41.49a	17.49	4.44c
Lavandin cv. grosso	39.30b	24.07b	10.84	8.25a
<b>Average</b>	<b>46.78</b>	<b>32.78</b>	<b>14.16</b>	<b>6.34</b>
Different letters indicate significant differences at the 0.05 level; ns: nonsignificant differences. GAE - gallic acid equivalent, CAE - cathetin acid equivalent.				

The findings show that the level of these bioactive components and the antioxidant capacity depended on the cultivar. The highest values of total phenolic, flavonoids, and antioxidant capacity were recorded in the lavender (54.26 mg GAE g<sup>-1</sup>, 41.49 mg CAE g<sup>-1</sup> and 17.49 μM Fe<sup>2+</sup> g<sup>-1</sup>, respectively), while the lowest was in the lavandin (39.30 mg GAE g<sup>-1</sup>, 24.06 mg CAE g<sup>-1</sup> and 10.84 μM Fe<sup>2+</sup> g<sup>-1</sup>, respectively). The observed differences between lavender and lavandin in the level of total phenolics can probably be attributed to genetic variations of the plant (Gavrić *et al.*, 2023). Our results align with Bajalan *et al.* (2016), which also researched flavonoid content in 30 different lavender cultivars. Their research showed that the content of flavonoids ranged from 28.19 to 71.62 mg GAE CAE g<sup>-1</sup> dry matter. Furthermore, the authors emphasize that it is crucial to identify lavender cultivars with a high content of flavonoids due to their positive pharmacological effects on human health. Furthermore, our results indicate that the highest antioxidant capacity was found in lavender than lavandin. Therefore, this suggests that the antioxidant capacity significantly

depends on the cultivars. In the meantime, Dobroš *et al.* (2022) studied three cultivars of lavender and two cultivars of lavandin.

They found that the cultivar greatly influences the antioxidative capacity activity. In addition to cultivars, environmental conditions such as temperature, humidity, altitude, location, and agronomic practices can significantly impact antioxidant capacity (Gavrić *et al.*, 2018a).

The quality of lavender and lavandin mainly depends on the essential oil yield and its content of constituents (Sařata *et al.*, 2020). Our research showed that the studied cultivars have a relatively high essential oil content. Also, analysis of essential oil content in our research showed a significant difference between the tested cultivars. The highest essential oil content was recorded in the lavandin (8.25 mL 100 g<sup>-1</sup>), followed by the *Lavanda cultivars* (4.44 mL 100 g<sup>-1</sup>). According to Mkaddem Mounira *et al.* (2022) variations in essential oil content can result from genetic factors, developmental stages, environmental conditions, harvesting methods, methods of drying, extraction, and analysis. Given that in our research, all factors that can influence the oil content were uniform, except for the cultivar, the resulting differences can be attributed to researched cultivars. This opinion can be supported by the finding of Renaud *et al.* (2001), who found that the essential oil content varies between 2.8-5.0% in lavender and 7.1-9.9% in lavandin.

Another important property that determines the quality and market price of essential oil of lavender and lavandin is the content of constituents. A data from the chromatogram (Figure 1 and 2) show that 23 different ingredients were isolated in essential oil of lavender and lavandin, and their percentage content is presented in Table 2.

The total of 23 ingredients were identified in the isolated essential oil, which makes up 98.06-98.30%. Furthermore, it is important to note that lavender and lavandin essential oils were different. In essential oil of lavender main isolated constituents were linalool (64.47%),  $\beta$ -cis-ocimene (6.50%), terpinen-4-ol (4.77%), linalyl acetate (4.22%),  $\beta$ -myrcene (3.38%), and other compounds in a smaller proportion. The main constituents in the essential oil of *Lavandin cv. grosso* were linalool (35.40%), eucalyptol (13.16%), camphor (9.54%), linalyl acetate (6.58%),  $\beta$ -myrcene (6.05%), lavandulol acetate (4.63%),  $\beta$ -cis-ocimene (4.56%), and other compounds.

Overall, our research determined that linalool is the main constituent of the essential oils of both cultivars. Therefore, by comparing this constituent between cultivars (Table 2), it can be said that lavender's highest proportion was recorded (64.47%). In contrast, lower linalool content was recorded in lavandin (35.40%). Our results are consistent with Lesage-Meessen *et al.* (2015), who also found higher linalool content in lavender than in lavandin cultivars.

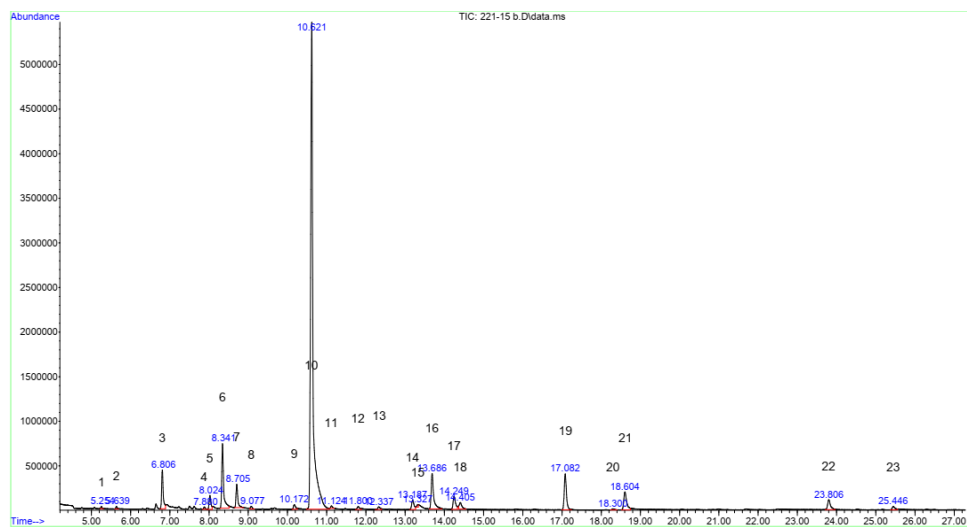


Figure 1. Chromatogram of lavender essential oil. Numbers of constituents: 1-  $\alpha$ -pinene, 2- camphene, 3-sabinene, 4- $\beta$ -pinene, 5- $\beta$ -myrcene, 6-2-carene, 7- m-cymene, 8-D-limonene, 9- eucalyptol, 10- $\beta$ -cis-ocimene, 11- trans-  $\beta$ -ocimene, 12-  $\gamma$ -terpinen, 13- terpinolen, 14- linalool, 15-camphor, 16-endo-borneol, 17-terpinen-4-ol, 18- $\alpha$ -terpineol, 19- linalyl acetate, 20-lavandulol acetate, 21- $\alpha$ -terpinyl acetate, 22-caryophyllene, and 23-cis- $\beta$ -farnesene.

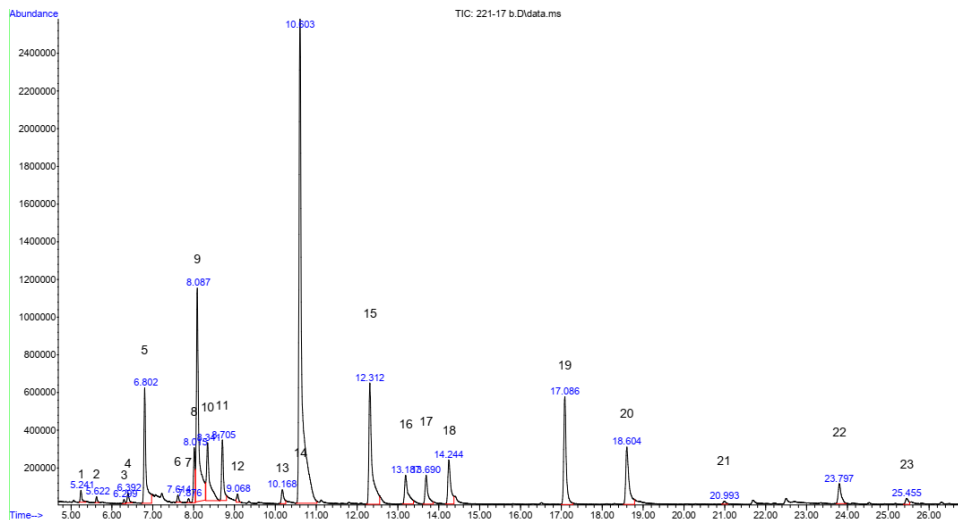


Figure 2. Chromatogram of lavandin essential oil. Numbers of constituents: 1- $\alpha$ -pinene, 2-camphene, 3- $\beta$ -myrcene, 4-m-cymene, 5-D-limonene, 6- $\beta$ -cis-ocimene, 7-trans-  $\beta$ -ocimene, 8- $\gamma$ -terpinen, 9-terpinolen, 10-linalool, 11-1-octen-3-yl-acetate, 12-cosmene, 13-camphor, 14-endo-borneol, 15-lavandulol, 16-terpinen-4-ol, 17- $\alpha$ -terpineol, 18-n-hexyl butanoate, 19-linalyl acetate, 20-bornyl acetate, 21-lavandulol acetate, 22-caryophyllene, and 23-cis- $\beta$ -farnesene.

Table 2. Chemical composition of essential oil lavender and *lavandin cv grosso*

№	Constituent name	Lavander essential oil, %	Lavandin cv. Grosso essential oil, %
1.	$\alpha$ - pinene	0.14	0.51
2.	camphene	0.18	0.21
3.	sabinene		0.16
4.	$\beta$ -pinene		0.48
5.	$\beta$ -myrcene	3.38	6.05
6.	2-carene		0.30
7.	m-cymene	0.17	0.16
8.	D-limonene	1.35	2.18
9.	eucalyptol		13.16
10.	$\beta$ -cis-ocimene	6.50	4.56
11.	trans- $\beta$ -ocimene	2.31	3.00
12.	$\gamma$ -terpinen	0.25	0.37
13.	terpinolen	0.54	0.84
14.	linalool	64.47	35.40
15.	1-octen-3-yl-acetate	0.20	
16.	cosmene	0.38	
17.	camphor	0.35	9.54
18.	endo-borneol	1.18	2.48
19.	lavandulol	0.18	
20.	terpinen-4-ol	4.77	2.07
21.	$\alpha$ -terpineol	1.73	3.18
22.	n-hexyl butanoate	0.93	
23.	linalyl acetate	4.22	6.58
24.	bornyl acetate	0.17	
25.	lavandulol acetate	2.85	4.63
26.	$\alpha$ -terpinyl acetate		0.19
27.	caryophyllene	1.53	1.56
28.	cis- $\beta$ -farnesene	0.51	0.46
	$\Sigma$ (%)	<b>98.29</b>	<b>98.06</b>

However, in our research, the content of linalool in lavender essential oil was slightly higher than the mentioned authors' results. According to Alizadeh and Aghaee (2016) these differences may arise due to cultivars, environmental conditions, harvest time, essential oil extraction processes, and determination methodology. In addition to the linalool content, linalyl acetate is a crucial constituent determining the quality of lavender essential oil (Pokajewicz *et al.*, 2021). In our research, its content ranged from 6.58% (*Lavandin cv. grosso*) to 4.22% (lavender). However, compared to previous studies (Pokajewicz *et al.*, 2021), this study found a relatively small amount of linalyl acetate. This difference can be attributed to partial decomposition during hydrodistillation (Danh *et al.*, 2013). Furthermore, the analyzed essential oils also differed in their camphor content. Lavender had a concentration of 0.35% camphor, while

lavender had a concentration of 9.54%. It should also be noted that a significant difference between the analyzed essential oils existed in the eucalyptol content. This constituent was found in significant concentrations in lavender essential oil (13.16%), while eucalyptol was not identified in lavender essential oil (0%). According to Kara and Baydar (2013) lavender essential oil is of higher quality in the perfume industry than Lavender essential oil because of its low camphor and eucalyptol level. The same authors believe that the camphor content in quality lavender oil must be between 0.5 and 1% in lavender and between 5.0 and 10.0% in lavender.

## CONCLUSIONS

This is the first research on lavender and lavandin grown in the environmental conditions of central Bosnia and Herzegovina. Research has shown that lavender has a high content of phenolic compounds, which is especially interesting if it is a source of antioxidants in the human diet. Furthermore, the research cultivars had a high yield of essential oil. Linalool dominated in the essential oil of both cultivars, especially lavender. The essential oil of lavender was rich in linalool and  $\beta$ -cis-ocimene but poor in camphor. On the hand, lavandin essential oil was rich in linalool, eucalyptol and camphor.

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