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INVESTIGATIONS ON THE BIOACTIVE COMPOUNDS AND ORNAMENTAL PROPERTIES OF SOME LAVENDER CULTIVARS

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Abstract

Lavandula angustifolia L. is a perennial plant with multiple uses in the cosmetic, pharmaceutical, food, aromatherapy industry and for ornamental purposes. Six lavender cultivars were used for measurements, laboratory analyses and decorations: L. angustifolia 'Hidcote', L. angustifolia 'Sevstopolis', L. angustifolia 'Nana Alba', L. angustifolia 'Dwarf Blue', L. angustifolia 'Blue Scent', Lavandula x intermedia 'Grosso', grown under field conditions. The ATR-FTIR spectra of various tissues of lavenders used in this experiment have revealed presence of a wide range of biochemical compounds with pharmaceutical importance. The content of lycopene, β-carotene, flavonoids, tannins and the antioxidant activity of each cultivar were determined, using UV-Vis spectroscopy. The best antioxidant activity has been noted in the 'Sevtopolis' cultivar, the 'Blue Scent' has the highest percentage of lycopene (3.47 mg/100 g) and the 'Dwarf White' cultivar the large amount of β-carotene (3.25 mg/100 g). The ornamental qualities have been highlighted by making decorations to create a relaxing space. The data obtained will help to identify the cultivars with the most biologically active principles as well as those with special decorative qualities.

Key words*: Lavender, UV-Vis spectroscopy, ATR-FTIR spectroscopy, ornamental, bioactive compounds.*

INTRODUCTION

The *Lamiaceae* family incorporates a wide variety of plants with biological and medical applications (Uritu et al., 2018). The genus *Lavandula* is an important member of this family comprising 39 species, 30 subspecies and varieties, as well as 17 hybrid taxa (Upson and Andrews, 2004). Lavender is cultivated mainly for its essential oil, with an important role in the pharmaceutical, cosmetic, perfumery and aromatherapy industries (Zuzarte et al., 2009; Prusinowska and Śmigielski, 2014; Tardugno et al., 2019, Haban, 2023). Studies have also confirmed its rich content in phenolic compounds, with strong antioxidant effects (Gallego et al., 2013; Piskernik et al., 2023; Chianese et al., 2023; Batiha et al., 2023).

The main phenolic compounds of lavender flowers are: hydroxybenzoic acids, hydroxycinnamic acids and flavonoids (Dobros et al., 2022), but new phenolic compounds such as lavandunat, lavandufurandiol, lavandufluoren lavandupyrones A and B, lavandudiphenyls A and B were isolated by Yadikar et al. in 2018. The level of bioactive compounds depends on the species, cultivars, geographical origin, climatic conditions, harvest time and extraction method (Dobros et al., 2022).

The most important cultivated species of the *Lavandula* genus are: *L. angustifolia* Mill.; *L. latifolia*.; *L. lanata* and *L. x intermedia* (a sterile hybrid obtained by crossing *L. angustifolia* and *L. latifolia*). The genus also includes numerous subspecies and several hundred cultivars (Passalacqua et al., 2017), very valuable from an ornamental point of view. They are distinguished by their elegant appearance, foliage in contrasting colours, fragrant flowers in distinct shades (white, light pink, shades of blue and purple) and adaptability in the landscape (Bader, 2012; Demasi et al., 2021). Fresh or dried flowers are used in floral art and in making potpourris to flavour interiors. Lavender extracts and essential oils with phytotoxic and insecticidal properties may also be valuable in the agrochemical industry (Pavela, 2005). In addition to the aesthetic and utilitarian aspects, lavender also has an important role in attracting beneficial insects, such as bees and butterflies, being a melliferous plant (Benachour, 2017). It can also add more flavour to syrups, drinks and

sweets. All these properties cause a significant increase in interest in the cultivation of lavender, both from an economic and medicinal perspective, as well as from an ecological and ornamental perspective. This study focuses upon the phytochemical profile of lavender extract, involving the flavonoids, tannins, carotenoids content and antioxidant activity. Moreover, our study aims to highlight the aesthetic value of some lavender cultivars as well as their role in alternative therapies.

MATERIALS AND METHODS

The study was carried out on six varieties of lavender grown in the field, in Argeș county. The mother plants were purchased from specialized companies. Specific care works were applied (watering, soil mobilization, shortening of branches to 15 cm every spring, fertilization with natural fertilizers 2 times/ year), for 3 years. In the 3rd year, the plants developed a uniform bush. Leaves and flowers were collected to determine the content of some biochemical compounds by ATR-FTIR and UV-Vis spectroscopy.

Lavandula angustifolia 'Sevtopolis'. Tall bush (50-70 cm), light blue-violet flowers, fragrant. High content in biochemical components, long flowering period and resistance to drought and frost. Suitable for landscaping, floral decorations, essential oil and floral water.

Lavandula angustifolia 'Hidcote'. Medium bush (40-60 cm), dark purple flowers, fragrant, blue-green foliage. It is a perfect choice for low hedges, borders, various decorations, dried flowers, as the flowers retain their colour and fragrance.

Lavandula angustifolia 'Nana Alba'. Attractive cultivar, flowering, with fragrant white flowers. It grows as a compact bush, low waisted (30 and 40 cm). It can be grown in containers, in beds, on terraces, slopes, borders.

Lavandula angustifolia 'Dwarf Blue'. Grows up to 20-25 cm, dense bush, gray-green leaves, purple inflorescences, fragrant. Tolerates partial shade and is drought tolerant. It is recommended for all types of gardens, in beds, borders, as cut flowers.

Lavandula angustifolia 'Blue Scent'. Compact shrub (40-50 cm), with gray-green leaves and slender, straight stems bearing fragrant inflorescences of blue flowers. Prefers sun exposure, well-drained soil. Drought tolerant. Good for borders, slopes, containers, gardens, urban areas, floral arrangements, rustic cottages. Good for drying and flavoring food and drinks.

Lavandula x intermedia 'Grosso'. Tall and vigorous cultivar (60-90 cm), with large and rich deep purple flowers, up to 15 cm long, strongly scented. It lends itself to decorations, as the flowers retain their fragrance and colour when dried. Excellent for all types of gardens.

Determination of the total content of tannins

The tannin content was determined with the methodology proposed by Cosmulescu et al. (2023), with some modifications. For analysis, a volume of 1 mL of aqueous extract was added to a 10 mL flask containing 2 mL of distilled water and 2 mL of Folin-Ciocalteu reagent. After 5 minutes, 5 mL of 10% sodium carbonate solution was added. After 60 minutes of rest, the absorbance of samples was measured at 760 nm and the concentration of tannins was expressed in mg GAE/100 g dry weight of plant material.

Determination of total content of flavonoids

A volume of 1 mL of methanolic extract was added to a 10 mL volumetric flask containing 4 mL of distilled water and 0.3 mL of 5% sodium nitrite. The mixture was allowed to stand 5 minutes, then 0.3 mL of 10% aluminium chloride was added to the volumetric flask. After other 5 minutes of rest, a 2 mL solution of 1 M sodium hydroxide was added and the volume of the sample was adjusted with distilled water to 10 mL. The absorbance of the solution was measured at 510 nm. Total flavonoid content was expressed as mg catechin equivalents per 100 g (mg CE/100 g).

Determination of carotenoids content

The concentration of carotenoids expressed as mg/100 g plant material was determined in the resulting supernatant according to the mentioned procedure by Cosmulescu et al. (2023). The molar extinction coefficients of 184900/M cm at 470 nm and 172000/M cm at 503 nm for lycopene and 108427/M cm at 470 nm and 24686/M cm at 503 nm for β-carotene in hexane were used.

Total antioxidant activity

Total antioxidant activity was determined using the methodology suggested by Lazar et al. (2020) and the results were expressed as a percentage of the inhibition of 2,2-diphenyl-1-picrylhydrazyl (DPPH I%).

Statistical Analysis

All analyses were performed in triplicate and data were reported as mean ± standard deviation (SD). Results were processed by Excel (Microsoft Office 2010) and SPSS Trial Version 28.0 (SPSS Inc., Chicago, IL, USA).

Data were subjected to analysis of variance (one-way ANOVA; $p \leq 0.05$), and Duncan's Multiple Range Test (DMRT) post hoc tests were used to measure specific differences between sample means. The Pearson correlation coefficient was used to measure the strength of the linear correlation between the determined parameters.

ATR-FTIR Analysis

The spectral measurements were made with a FTIR Jasco 6300 spectrometer. An ATR accessory equipped with a diamond crystal (Pike Technologies) allows collection of FTIR spectra directly on a sample without any special preparation. The spectra were recorded in the region of 4000-400 cm^{-1}, detector TGS, apodization Cosine. The spectral data were processed with JASCO Spectra Manager II software. Samples were scanned at 4 cm^{-1} resolution, accumulation: 100 scans. IR bands were identified by comparison with published assignments (Silverstein and Webster, 1998; Mossoba et al., 2005; Movasaghi et al., 2008; Munteanu et al., 2023). The crystal was cleaned between measurements with ethanol and dried with a lint-free tissue. For each sample, three or four spectral data were performed to ensure the spectral reproducibility and assess analytical precision and the average spectrum was done.

Chemometric Analysis

Infrared Spectra were exported from Spectra Manager, in ASCII (dx) format, into the Unscrambler Software (Edition X 10.4, Camo Oslo Norway) for chemometric analysis. Spectra were pre-processed using the second-derivative transformation, the Savitzky-Golay derivation. The use of spectra derivatives with the Savitzky-Golay algorithm as a chemo-metric pre-processing technique is widely reported in most classifications based on FTIR spectroscopy (Dovbeshko et al., 2000; Topală and Tătaru, 2018; Topală et al., 2020; Vîjan et al., 2023).

The principal component analysis (PCA) model was developed using cross-validation. PCA was performed both on the entire spectral range (4000 to 400 cm^{-1}) and on the MIR spectral region (1680-1400 cm^{-1}), Validation: Cross Validation, Algorithm: Singular Value Decomposition (SDV).

RESULTS AND DISCUSSIONS

Determination of tannins, flavonoids, carotenoids and antioxidant activity

The biochemical compounds of lavender flowers extract are represented in Table 1. According to the results obtained, the mean concentration of tannins was 1479.11 mg GAE/100 g, with a minimum content in 'Nana alba' cultivar (1372.20 mg GAE/100 g) and the maximum in 'Dwarf Blue' (1725.31 mg GAE/100 g). Our results are in line with Costea et al. (2019) that report 1325 mg GAE/100 g tannins for lavender flowers collected in June.

Table 1. The biochemical compounds of studied cultivars

Cultivar	Flavonoids (mg CE/100 g)	Tannins (mg GAE/100 g)	Lycopene (mg/100 g)	β-carotene (mg/100 g)	DPPH I%
'Blue Scent'	1305.91±11.73 b	1372.66±92.08 c	3.47±0.04 a	2.38±0.08 d	82.06±0.35 d
'Nana White'	764.57±6.75 d	1372.20±0.21 c	2.34±0.03 d	3.25±0.04 a	72.39±0.30 e
'Hidcote'	1302.24±7.20 b	1403.75±0.12 b	2.29±0.03 d	2.37±0.04 d	86.37±0.29 b
'Dwarf Blue'	1148.78±1.21 c	1725.31±0.50 a	1.21±0.02 e	1.56±0.04 e	85.89±0.27 bc
'Sevstopolis'	1247.83±4.60 b	1511.33±0.14 b	2.90±0.04 b	2.64±0.08 c	87.91±0.30 a
'Grosso'	1549.80±81.49 a	1489.41±0.13	2.82±0.04 c	3.03±0.10 b	85.63±0.36 c
Total	**1219.86±245.23**	**1479.11±130.06**	**2.51±0.72**	**2.54±0.56**	**83.37±5.38**
Min.	**760.53**	**1319.44**	**1.19**	**1.52**	**72.09**
Max.	**1598.06**	**1725.72**	**3.51**	**3.28**	**88.20**

The average flavonoid content was 1219.86 mg/ 100 g and variation limits between 764.57 mg/ 100 g ('Nana Alba') and 1549.80 mg/100 g ('Grosso').

Galego et al. (2013) reported that the lavender flowers lyophilized extract had a lower polyphenol content (52 ± 2.1 mg gallic acid/g) than the leaves. Radulescu et al. (2016) investigated the chemical composition of *Lavandula angustifolia* extracts obtained by different extraction methods. They reported a content of 78.345±0.982 (µg/mg total extract) total flavonoids obtained by subcritical fluid extraction.

Carotenoids showed oscillations from 1.21 to 3.47 mg/100 g (mean value of 2.51 mg/100 g) for lycopene and from 1.56 to 3.25 mg/100 g (mean value of 2.54 mg/100 g) for β-carotene.

According to the results obtained by Dobreva et al. in 2024, levels of carotenoids in lavender were higher in conventional farming (between 55.5 and 77.3 µg/g) than in organic farming (between 36.9 and 72.2 µg/g).

Measurements of antioxidant activity of lavender plant extracts showed a significant variation between cultivars. The highest antioxidant activity was determined in 'Sevstopolis' and the lowest in 'Nana Alba' cultivars (87.91 and 72.39 %, respectively).

In the study by Galego et al. (2013), the extracts from lavender leaves had the highest antioxidant activity, 1.5± 0.06 (mM Trolox/g lyophilized extract), followed by lavender stems and the lavender flowers 0.2±0.01 (mM Trolox/g lyophilized extract).

According to the data of Radu (Lupoae) et al. (2019), the lavender extract displayed a high polyphenolic and flavonoids content, with an antioxidant activity of 2.28 mmol Trolox/g. D.W. Komes et al. (2011) studied the phenolic composition and antioxidant activity of some medicinal plants, including lavender, as affected by the extraction time and hydrolysis and reported a higher content of 4.94 mg catechin/L in hydrolysed extract of lavender. Lee and Shibamoto (2002) observed low antioxidant activities of volatile extracts of lavender in comparison to thyme, basil and rosemary.

According to Costea et al. (2019) the antioxidant properties of lavender plants were in correlation with the contents of total phenolic compounds and flavonoids in extracts. As shown in Table 2, the antioxidant activity (DPPH I %) correlated positively very significantly with flavonoids (r=0.778***), positively significantly with tannins (r=0.469*), but showed a significant negative correlation with β-carotene (r=-0.521*). Also, there was an increase in tannin content distinctly significantly correlated with a reduction in lycopene content (r=-0.697**) and β-carotene (r=-0.684**). In the case of carotenoids, between lycopene and β-carotene there was a significant positive correlation (r=0.531*).

Table 2. Correlation matrix between the content of flavonoids, tannins, lycopene, β-carotene and antioxidant activity in the lavender cultivars (r values are presented)

		Flavonoids (mg CE/ 100 g)	Tannins (mg GAE/ 100 g)	Lycopene (mg/100 g)	β-carotene (mg/100 g)	DPPH I %
Flavonoids	Pearson Correlation	1	0.115	0.350	-0.157	0.778***
	Sig. (2-tailed)		0.649	0.155	0.535	0.000
Tannins	Pearson Correlation		1	-0.697**	-0.684**	0.469*
	Sig. (2-tailed)			0.001	0.002	0.049
Lycopene	Pearson Correlation			1	0.531*	-0.041
	Sig. (2-tailed)				0.023	0.870
β-carotene	Pearson Correlation				1	-0.521*
	Sig. (2-tailed)					0.026
DPPH I%	Pearson Correlation					1
	Sig. (2-tailed)					

***. Correlation is significant at the 0.001 level (2-tailed).
**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

ATR-FTIR Analysis

Leaves are complex assemblages of organic compounds and it might be expected that they would display distinctive spectral features in the infrared range (4000-400 cm^{-1}). Fundamental vibration modes of various

molecular functional groups produce characteristic spectral absorption features that can serve to fingerprint many compounds (Vîjan at al., 2023). Such functional groups and related spectral features include hydroxyl (OH) in alcohols and acids, carbonyl (C=O) in esters, ketones, aldehydes and acids, and methyl (CH_3) and methylene (CH_2) in alkanes.

To compare the lavender samples FTIR spectroscopy was used as an efficient method. Figure 1 shows the ATR-FTIR spectra of the lavender samples with major high bands and highlights the ornamental potential depending on biometric characteristics and aesthetics qualities.

Typically, the characteristic differences in the FTIR spectral analysis for lavender samples were observed (Tables 3 and 4).

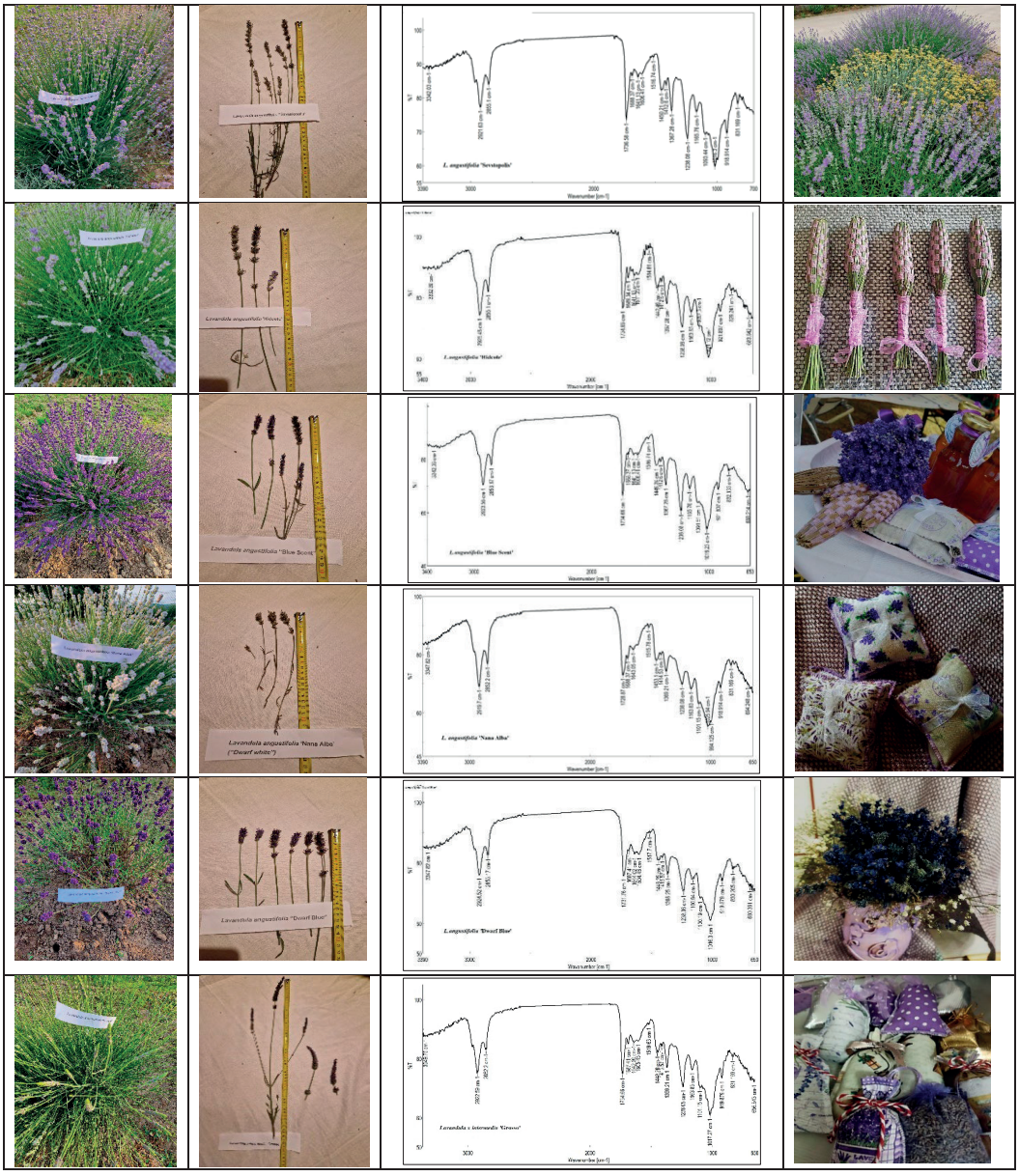

Figure 1. The ATR-FTIR spectra of the lavender samples

Table 3. ATR-FTIR spectra vibrational assignments for lavender flowers

Frequencies [cm^{-1}]						
'Hidcote'	**'Sevstopolis'**	**'Nana Alba'**	**'Dwarf Blue'**	**'Blue Scent'**	**'Grosso'**	**Peak assignment**
3332	3343	3347	**3347**	3342	3349	Stretching O-H, N-H, C-H
2925	2921	2919	2924	2923	2922	Asymmetric stretching vibration of CH_2
2855	2855	2852	2853	2853	2852	Symmetric stretching vibration of CH_2
1734	1736	1728	1731	1734	1734	νC=O stretching due to lipids
1689	1688	1688	**1687**	1688	1687	Stretching νC=O, Amide I
1658 1641	1641	**1643**	**1644**	1641	1642	Amide I band of protein and H-O-H deformation of water (Movasaghi et al., 2008)
1611	1606		**1604**	1606	**1605**	Adenine vibration in DNA (Mossoba et al., 2005)
1514	1516	1515	**1517**	1516	**1519**	Amide II
1442	1450	**1453**	**1446**	1449	1448	Asymmetric CH_3 bending of the methyl groups of proteins (Movasaghi et al., 2008)
1412	1412	**1414**	**1413**	1412	1413	Stretching C-N, deformation N-H, deformation C-H (Dovbeshko, 2000)
1367	1367	1369	**1368**	1367	1369	Stretching C-O, deformation C-H, deformation N-H (Dovbeshko, 2000)
1238	1238	1238	1238	1238	1238	Stretching PO_2^- asymmetric (phosphate I) (Dovbeshko, 2000)
1163	1165	1163	**1160**	1165	**1163**	Stretching ν_{as} O–C–O
1097	1093	**1101**	**1100**	1091	**1101**	Phosphate ester (C-O-P) band (Movasaghi et al., 2008)
1021	1016	**1025**	1016	1018	1017	ν(CO), ν(CC), δ (OCH), ring (polysaccharides, pectin) (Movasaghi et al., 2008)
		994				C-O ribose, C-C (Movasaghi et al., 2008)
921	918	918	919	**921**	919	Phosphodiester region
829	831	831	830	832	831	$C_2^{'}$ endo conformation of sugar (Movasaghi et al., 2008)
683	692	694	690	668	656	OH out-of-plane bend

Table 4. ATR-FTIR spectra vibrational assignments for lavender leaves

Frequencies [cm^{-1}]						
'Hidcote'	**'Sevstopolis'**	**'Nana Alba'**	**'Dwarf Blue'**	**'Blue Scent'**	**'Grosso'**	**Peak assignment**
3342	3351	3344	**3348**	3353	3344	Stretching O-H, N-H, C-H
2918	2917	2918	2918	2923	2917	Asymmetric stretching vibration of CH_2
2850	2851	2850	2850	2853	2849	Symmetric stretching vibration of CH_2
1728	1727	1727	1728	1730	1726	νC=O stretching due to lipids
1687	1690	1690	**1687**	1686	1688	Stretching νC=O, Amide I
1643	1658	1658	**1643**	1641	1642	Amide I band of protein and H-O-H deformation of water (Movasaghi et al., 2008)
1605	1604	**1610**	**1602**		**1612**	Adenine vibration in DNA (Mossoba et al., 2005)
1516	1514	1514	**1516**	1516	**1515**	Amide II
1454	1442	**1453**	**1453**		1453	Asymmetric CH_3 bending of the methyl groups of proteins (Movasaghi et al., 2008)
1413	1412	**1411**	**1414**	1414	1413	Stretching C-N, deformation N-H, deformation C-H (Dovbeshko, 2000)
1368	1368	1368	**1368**	1366	1367	Stretching C-O, deformation C-H, deformation N-H (Dovbeshko, 2000)
1316				**1315**	**1314**	Amide III band components of proteins
1237	1237	1234	1237	1238	1236	Stretching PO_2^- asymmetric (phosphate I) (Dovbeshko, 2000)
1142	1145	1167	1162	1144	**1169**	Stretching ν_{as} O–C–O
			1142			Phosphate & oligosaccharides Oligosaccharide C-O bond in hydroxyl group that might interact with some other membrane components Membrane-bound oligosaccharide C-OH bond (Movasaghi et al., 2008)
1093	1095	1096	**1098**	1096	**1100**	Phosphate ester (C-O-P) band (Movasaghi et al., 2008)
1008	1009	**1010**	1005	1006	**997**	ν(CO), ν(CC), δ (OCH), ring (polysaccharides, pectin) (Movasaghi et al., 2008)
		891		**894**	892	Phosphodiester region
		833	831	832	831	$C_2^{'}$ endo conformation of sugar (Movasaghi et al., 2008)
755 654	800 750	800 754 661	650	799 724 635	765 732 651	OH out-of-plane bend

For the lavender samples considered, the first three principal components (PCs) represent 100% for flowers (PC1= 96%, PC2= 6%, and PC3 = 1%), 99% respectively for lavender leaves. This indicates that these three components were provided a good separation between the groups (Figures 2 and 3).

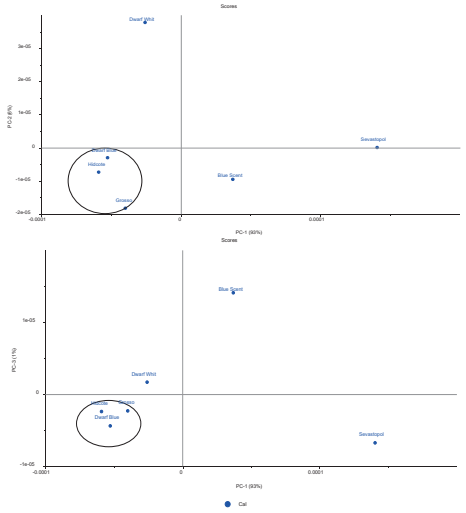

Figure 2. 2-D scores obtained from PCA of FTIR spectra of lavender flower samples for the first two PCs a), and PC3 versus PC1 b).

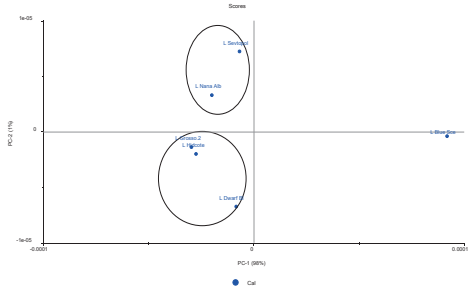

Figure 3. 2-D scores obtained from PCA of FTIR spectra of lavender leaves samples for the first two PCs

Principal component analysis (PCA) includes the region at 1680-1400 cm^{-1}. 'Hidcote', 'Dwarf Blue' and 'Grosso' were separated from the other samples, both in leaves and flowers.

Both the flower stalks and the flowers from the inflorescences, fresh and dry, were used to make specific decorations for a therapeutic space.

CONCLUSIONS

The obtained results revealed a high content of flavonoids, tannins, lycopene and β-carotene in 'Sevstopolis', 'Grosso', 'Hidcote' and 'Dwarf Blue' in contrast to 'Nana Alba' and 'Blue Scent'. The antioxidant activity was correlated positively very significantly with flavonoids and positively significantly with tannins. The lavender flower extract for 'Nana Alba' with low antioxidant activity exhibited relatively low flavonoid content. 'Sevastopolis' cultivar had the highest antioxidant activity, therefore it is the most frequently used in the phytopharmaceutical industry.

ATR-FTIR spectra coupled with chemometric analysis can be effectively used to discriminate lavender species. In our study, 'Grosso', 'Dwarf Blue' and 'Hidcote' were separated by chemometry from the FTIR analysis; they also had a high content of tannins and flavonoids, a fact confirmed by the UV-VIS spectral analysis.

The varieties 'Nana Alba', 'Blue Scent', 'Dwarf Blue' are easy to integrate into the landscape thanks to their elegant port. 'Dwarf Blue' and 'Blue Scent' floral stems are used in floral art as accent points, keeping their colour alive even after drying. 'Grosso' offers the largest floral mass and together with 'Sevstopolis' and 'Hidcote' are suitable for potpourri, flower arrangements, scented bags and small pillows as the flowers retain their fragrance for a long time.

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