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Essential oil composition and antioxidant activities of eight cultivars of Lavender and Lavandin from western Anatolia

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ABSTRACT

The aim of this study was to examine the essential oil compositions and antioxidant activities of six *L. angustifolia* cultivars and two *L. x intermedia* in Turkey. The chemical composition of essential oils obtained by steam-distillation from fresh flowers of *Lavandula* samples were analyzed using gas chromatography-mass spectrometry (GC/MS). Antioxidant activities of lavender and lavandin cultures were evaluated using β-Carotene Bleaching, DPPH· and ABTS·$^+$ assays. Sixty-six components were identified for all cultivars. The results indicate that there is a significant difference between *L. angustifolia* and *L. x intermedia* cultivars in terms of major components, linalool, linalyl acetate. Linalyl acetate (%46.887–%29.098) and linalool (%36.801–%28.102) were determined in six the cultivar of *L. angustifolia* and Super A cultivar of *L. x intermedia*. Linalool (%28.486) and eucalyptol (%15.650) were found as abundant in *L. x intermedia* Grey Hedge. Evaluation of antioxidant activity of the studied samples emphasizes that highest inhibition was observed in *L. angustifolia* Yubileina cultivar (23.67 ± 0.14 μg mL^{-1}). However, in the DPPH· assay, *L. x intermedia* Super A cultivars showed the highest inhibition activity (IC_{50}) 89.81 ± 0.17 μg mL^{-1}. In the ABTS·$^+$ assay, *L. angustifolia* Sevtopolis cultivar displayed highest radical scavenging activity with inhibition values of 61.23 ± 0.11 μg mL^{-1}. Essential oil composition of eight lavender and lavandin varieties, used in the industry, was analyzed. And there is a significant difference in terms of camphor composition. The obtained data have been inquired by principal components analysis (PCA), allowing differentiation of eight lavender and lavandin cultures by their variety origins. High levels of linalyl acetate and linalool, low level of camphor (< 0.5%) and high antioxidant activity shown that, these cultivars may be considered as a natural raw material source for pharmaceuticals and cosmetic products.

1. Introduction

Medicinal and aromatic plants have been used for variety of aims since ancient times and recently have a strong position as economical crops around the world for essential oil production (Bajalan et al., 2016). Nowadays, these plant essential oils have become commercially popular due to their impression as a “well-being” life style (Yang et al., 2010).

The industrial cultivation and production of *Lavandula angustifolia* Mill. and *Lavandula x intermedia* Emeric as medicinal and aromatic plants have been rapidly raised during the last years and the World’s interest for *Lavandula* essential oil is still increasing. Therefore, detailed analyses of produced essential oils to figure out their quality and quantity are highly important for the selection of industrial usage. The trade value of essential oil export in the world is approximately 1.90–2.00 billion dollars and about 50 million dollars of this currency belong to *Lavandula* essential oil (Gökdoğan, 2016).

Essential oils, obtained from medicinal and aromatic plants by using various methods such as steam distillation, hydro distillation, cold press or extraction, are mixtures of various chemical constituents including terpenes, alcohols, aldehydes, phenols and esters, which may produce significant fragrances (Grassmann and Elstner, 2003; Ali et al., 2015).

The genus *Lavandula* (*Lamiaceae* family) is one of the most well-known essential oil crop in the world with its 39 species, numerous hybrids and about 400 officially registered cultivars (Benabdelkader et al., 2011). The main producing regions are Europe, the Middle East, Asia and Northern Africa. France and Bulgaria. Those dominate the production but also Morocco, Yugoslavia, Hungary, Italy, Russia, Spain, Romania, Ukraine and Turkey have production in different amounts (Zheljazkov et al., 2013).

Many *Lavandula* species have essential oils with aromatic and medicinal properties that able use in the cosmetic, pharmaceutical and food industries (Torras-Claveria et al., 2007) but specially three of these species are important with their high commercial value: Lavender

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(*Lavandula angustifolia* Mill. syn. *L. officinalis* Chaix ex Vill syn. *L. vera* DC syn. *L. spica* (true lavender, fine lavender or English lavender), Lavandin (*Lavandula intermedia* Emeric ex Loisel syn. *L. hybrid,* a hybrid of *L. angustifolia* and *L. latifolia*), and Spike lavender (*Lavandula latifolia* Medicus) (Lesage-Meessen et al., 2015). The world production of lavender oil is approximately 200 tons per year. Bulgaria, UK and France are dominating the lavender essential oil production. The world production of lavandin oil is about 1200 tons per year with a rate of 90% representing by France (Karapandzova et al., 2012).

The essential oils of the *Lavandula* species have the same chemical composition but these components are present in different proportions. Oil composition and oil yield of *Lavandula* differentiate each other. Common criteria for the determination of oil quality are camphor, linalool and linalyl acetate levels of essential oil (Baydar and Kineci, 2009). According to the ISO 3515:2002 standard, lavender essential oil contains linalool (25–38%), linalyl acetate (25–45%) and camphor (0.5–1.0%), lavandin essential oil contains linalool (24–35%), linalyl acetate (28–38%) and camphor (6–8%) according to the ISO 8902:2009.

Due to these specifications, *lavandula* essential oil is used in food manufacturing industry as flavoring agent, preservative additives for cosmetics and fragrance industry including soaps, colognes, perfumes, skin lotions (Da Porto et al., 2009; Muyima et al., 2002; Kunicka-Styczyńska et al., 2009; Fakhari et al., 2005). Paricularly, while Lavender essential oil is used in industrial areas such as perfumery, pharmaceuticals and cosmetic due to its high linalool and linalyl acetate content, lavandin essential oil is commonly used in hygiene products, industrial and household cleaner products, detergents and insecticides due to high levels of camphor (Cavanagh and Wilkinson, 2002; Lesage-Meessen et al., 2015). Additionally, altough lavandin essential oil is produced in higher yields than lavender essential oil (120 kg/ha, 40 kg/ha, respectively) (Carrasco et al., 2016a; Kara and Baydar 2013), lavender essential oil quality and price (85–150€/kg) is higher than lavandin essential oil (19€/kg) (Lesage-Meessen et al., 2015).

Lavandula essential oil, with the main active constituents linalool, linalyl acetate, 1,8-cineole, *cis* and *trans*-ocimene, terpinen-4-ol and camphor, has been reported to have antimicrobial, anticholinesterase and antioxidant activities (Costa et al., 2012; Hanamanthagouda et al., 2010; Cavanagh and Wilkinson, 2002; Gonçalves and Romano, 2013). Thus, *Lavandula* oil promotes healing symptoms for stress, exhaustion, migraines, anxiety, insomnia and depression (Rafie et al., 2016; Danh et al., 2013; Fismer and Pilkington, 2012; Koulivand et al., 2013).

When we consider all the pharmacological properties and the rich chemical content, *lavandula* essential oil is a significant product and encourage the cultivation of this plant as an industrial crop for essential oil production (Stanev et al., 2016; Adal et al., 2015).

In the literature, there is a lot of research dedicated to essential oil compositions and antioxidant activities of *L. angustifolia* species (Hanamanthagouda et al., 2010; Costa et al., 2012; Kiran Babu et al., 2016; Fakhari et al., 2005), its Bulgarian cultivars (Zagorcheva et al., 2013; Baser et al., 2005; Stanev et al., 2016) and *L. x intermedia* (Bajalan et al., 2016; Blazekovic et al., 2010; Carrasco et al., 2016b; Erbaş and Baydar, 2008) but there is no comperative study on chemical compositon and antioxidant activites of *L. angustifolia* Mill. ("Sevtopolis", "Yubileina", "Druzhba", "Raya", "Hebar", "Hemus") and *L. x intermedia* ("Super A", "Grey Hedge") essential oils in Turkey.

The aim of this study to investigate the essential oil composition and antioxidant activity of *L. angustifolia* cultivars ("Sevtopolis", "Yubileina", "Druzhba", "Raya", "Hebar", "Hemus") and *L. x intermedia* cultivars ("Super A", "Grey Hedge") in Turkey and encourage the breeder to growth of this industrial crops by showing their quality specifications and possible commercial value.

2. Materials and methods

2.1. Plant material

Whole plant material of six *Lavandula angustifolia* Mill. cultivars including "Sevtopolis", "Yubileina", "Druzhba", "Raya", "Hebar", "Hemus" were collected from Fethiye, Kabaağaç (36°33′45.80" N–29°17′21.07" E), *Lavandula x intermedia* var. Grey Hedge from Fethiye, Göcek, Gökçeovacık (36°47′11.67" N–28°58′51.17" E), and *Lavandula x intermedia* var. Super A were collected from Burdur, Akçaköy (37°42′28.14" N–29°52′37.94" E) Turkey in June 2016 when the crop was full of blossom. The spices were identified from Muğla Sıtkı Koçman University, Faculty of Science, Department of Molecular Biology and Genetics. The plant materials were studied fresh.

2.2. Isolation of the essential oil

Aerial parts of freshly harvested plants were immediately subjected to steam distillation for 2 h to extract the essential oil. The resulting oil was dried with anhydrous sodium sulphate and stored in an amber bottle at +4 °C in a refrigerator until time of analysis.

2.3. GC/MS analysis

GC/MS analyses were carried out using an Agilent 6890N Gas Chromatograph equipped with a split/splitless injector (200 °C), a DB-1MS capillary column (30 m × 0.25 mm; film thickness 0.25 μm) and coupled with an Agilent 5975C MS Detector, operating in the electron impact (EI) mode at 70 eV. Transfer line temperature was set at 250 °C. The carrier gas was He (2.6 mL min^{-1}), and the oven temperature was programmed from 60 °C to 280 °C at a rate of 3 °C/min. The injected volume was 1 μL and the split ratio 50:1.

The identification of the compounds was based on the comparison of their retention times (RT) and mass spectra with those from the NIST and Wiley 2008 libraries. Relative percentages of compounds were calculated based on the peak areas from their GC–MS chromatograms.

2.4. Antioxidant activity

The in-vitro antioxidant activity of Lavander and Lavandin essential oils were examined by three complementary methods, inhibition of β-carotene bleaching assay, DPPH radical scavenging activity and ABTS cation radical decolorization assay and results calculated with the same equations given by Kıvrak and Kıvrak (2014). α-Tocopherol were used as standard and all tests were done in triplicate.

2.4.1. Inhibition of β-carotene bleaching assay

The total antioxidant activity was determined using β-carotene-linoleic acid test method (Miller, 1971) based on the detection of inhibition of conjugated dien hydroperoxides because of oxidation of linoleic acid with slight modifications described by Kıvrak (2015). β-Carotene (0.5 mg), dissolved in 1 mL of chloroform, was mixed with linoleic acid (25 μL) and Tween 40 emulsifier (200 mg). Chloroform was evaporated under vacuum, 50 mL of distilled water saturated with oxygen was added by vigorous shaking. Aliquots (160 μL) of this emulsion were added to 40 μL of the extract solutions at different concentrations. As soon as the emulsion was added to each tube, the zero time absorbance was initially measured at 470 nm, and then the absorbance measurements were done for every 30 min until 120 min. The results were given as 50% inhibition concentration (IC_{50}). The sample concentration inhibiting 50% antioxidant activity (IC_{50}) was calculated from the graph of activity percentage against sample concentration. The antioxidant activity was calculated in terms of percent inhibition relative to the control, using eq. (1)

$$\text{Antioxidant activity (\%)} = (A_{Control} - A_{Sample})/A_{Control} \times 100 \quad (1)$$

Table 1
Percentages (%) of essential oil content of lavender and lavandin cultivars.

No	Essential oil	RI	LAS	LAY	LAD	LAR	LAHb	LAHm	LISA	LIGH
1	1-Hexanol	789	0.031	0.074	0.017	0.025	0.013	0.009	0.059	0.195
2	Tricyclene	918	0.016	0.040	0.004	0.019	0.006	0.004	0.018	0.053
3	α-Thujene	921	0.033	0.111	0.058	0.059	0.007	0.003	0.009	0.306
4	α-Pinene	930	0.094	0.259	0.056	0.200	0.025	0.015	0.244	1.527
5	Camphene	942	0.106	0.245	0.030	0.104	0.040	0.022	0.240	0.742
6	Sabinene	958	0.038	0.089	0.016	0.052	0.008	0.005	0.076	0.595
7	β-Pinene	960	0.084	0.065	0.013	0.077	0.011	0.016	0.147	1.628
8	3-Octanone	965	1.761	4.003	0.390	2.043	0.878	0.395	0.595	0.161
9	β-Myrcene	975	0.861	1.603	0.773	0.669	0.499	0.482	0.692	1.214
10	α-Phellandrene	989	0.036	0.060	0.043	0.037	0.039	0.023	0.037	0.194
11	Hexyl acetate	998	0.810	1.634	0.337	0.366	0.561	0.193	0.559	0.130
12	3-Carene	1004	0.105	0.209	0.048	0.106	0.100	0.029	0.034	0.405
13	o-Cymene	1008	0.048	0.077	0.075	0.034	0.035	0.015	0.033	0.314
14	p-Cymene	1009	0.096	0.172	0.082	0.053	0.047	0.029	0.055	0.364
15	Eucalyptol	1015	1.640	3.029	0.615	1.274	0.404	0.505	4.051	15.650
16	α-Limonene	1017	0.224	0.368	0.283	0.162	0.148	0.175	0.707	2.349
17	β-*trans*-Ocimene	1026	1.587	3.147	1.742	2.668	2.766	0.804	1.452	5.027
18	β-*cis*-Ocimene	1035	3.317	5.929	1.482	3.119	2.860	0.916	2.647	1.827
19	γ-Terpinene	1045	0.066	0.102	0.181	0.069	0.014	0.011	0.029	0.573
20	Sabinene hydrate	1048	0.054	0.081	0.026	0.055	0.020	0.009	0.027	0.200
21	*trans*-Linalol oxide	1053	0.137	0.207	0.177	0.116	0.197	0.096	0.159	0.072
22	1-Octanol	1055	0.037	0.038	0.037	0.018	0.019	0.026	0.112	0.193
23	*cis*-Linalol oxide	1067	0.101	0.150	0.135	0.082	0.150	0.075	0.121	0.069
24	α-Terpinolen	1074	0.065	0.063	0.207	0.065	0.081	0.105	0.252	0.642
25	*cis-beta*-Terpineol	1077	0.021	0.031	ND	0.015	ND	ND	ND	0.083
26	α-Naginatene	1079	ND	ND	ND	ND	0.031	ND	ND	ND
27	Linalool	1089	28.102	30.455	35.113	35.491	28.782	29.349	36.801	28.486
28	1-octen-3-ol, acetate	1095	0.808	1.181	0.582	0.519	0.536	0.571	0.175	0.008
29	Camphor	1117	0.385	0.309	0.185	0.209	0.384	0.203	5.261	6.355
30	Hexyl-2-methyl propanoate	1134	0.082	0.112	0.086	0.075	0.095	0.086	0.182	0.208
31	Borneol	1141	0.919	0.458	0.446	0.433	1.022	0.529	2.161	8.108
32	Cryptone	1145	0.169	0.090	0.122	0.081	0.148	0.149	0.107	0.204
33	Lavandulol	1149	0.861	0.697	0.656	0.675	0.344	0.227	0.145	0.464
34	Terpinen-4-ol	1156	1.081	0.883	3.693	1.661	0.187	0.065	0.036	6.880
35	Myrtenal	1161	ND	ND	ND	ND	ND	ND	ND	0.022
36	α-Terpineol	1168	0.844	0.338	2.236	0.635	0.632	1.575	1.145	0.998
37	Hexyl butyrate	1174	1.090	1.217	0.949	0.768	0.840	0.713	1.698	1.280
38	*cis*-Carveol	1186	0.005	ND	ND	ND	ND	0.012	0.010	0.016
39	Cumic aldehyde	1206	0.178	0.109	0.112	0.083	0.165	0.155	0.147	0.564
40	(+)-Carvone	1210	0.016	ND	0.007	ND	0.013	0.011	0.014	0.036
41	*cis*-Geraniol	1214	0.074	0.017	0.259	0.069	0.073	0.244	0.118	0.031
42	(+)-Piperitone	1220	0.012	ND	0.005	0.005	0.008	0.012	0.012	0.022
43	Hexyl 2-methyl butanoate	1227	0.082	0.073	0.081	0.056	0.083	0.047	0.188	0.493
44	*trans*-Geraniol	1240	0.137	0.024	0.653	0.156	0.120	0.539	0.319	0.068
45	Linalyl acetate	1244	32.121	29.098	33.297	33.923	45.593	46.887	33.087	4.648
46	Cumic alcohol	1258	0.029	ND	0.033	0.017	0.021	0.070	0.023	0.064
47	Bornyl acetate	1263	0.149	0.096	0.074	0.215	0.187	0.207	0.062	0.089
48	Lavandulol acetate	1272	4.840	3.386	2.607	4.337	3.416	3.922	1.230	0.831
49	Hexyl crotonate	1310	0.092	0.050	0.080	0.082	0.133	0.139	0.471	0.280
50	Nerol acetate	1341	0.151	0.045	0.506	0.160	0.176	0.491	0.249	0.040
51	Geranyl acetate	1360	0.207	0.054	0.898	0.252	0.305	0.918	0.459	0.084
52	α-Bourbonene	1362	0.133	0.112	0.126	0.083	0.045	0.027	0.102	0.068
53	Hexyl hexanoate	1370	0.180	0.100	0.305	0.055	0.106	0.093	0.122	0.294
54	α-Gurjunene	1373	ND	ND	ND	ND	ND	ND	ND	0.141
55	Zingiberene	1380	0.161	0.100	0.072	0.050	0.083	0.070	0.086	0.023
56	β-Caryophyllene	1406	6.290	4.205	4.784	4.266	2.745	3.514	1.545	0.637
57	α-Santalene	1410	0.381	0.247	0.319	0.233	0.226	0.169	ND	0.100
58	α-Bergamotene	1425	0.160	0.096	0.120	0.077	0.081	0.068	0.048	0.073
59	α-Caryophyllene	1438	0.227	0.124	0.190	0.141	0.102	0.131	0.039	0.022
60	β-Sesquiphellandrene	1447	0.110	0.049	0.062	0.053	0.060	0.037	0.018	0.040
61	β-Farnesene	1456	7.053	3.888	3.497	2.731	3.881	3.999	0.914	3.296
62	Germacrene-D	1464	1.119	0.508	0.544	0.764	0.257	0.134	0.372	0.185
63	γ-Cadinene	1509	0.102	0.029	0.050	0.013	0.017	0.121	0.039	0.026
64	Caryophyllene oxide	1557	0.228	0.058	0.378	0.136	0.167	0.347	0.050	0.043
65	α-Cadinol	1636	0.054	0.006	0.046	0.009	0.008	0.207	0.024	0.017
66	α-Bisabolol	1679	ND	ND	ND	ND	ND	ND	0.186	0.243

ND: not detected; RI: retention indices relative to C_6 – C_{20} n-alkanes on DB-1MS column, *L. angustifolia* cultivars (LAS: Sevtopolis, LAY: Yubileina, LAD: Druzhba, LAR: Raya, LAHb: Hebar, LAHm: Hemus) and *L. x intermedia* cultivars (LISA: Super A, LIGH: Grey Hedge).

where $A_{Control}$ is the initial concentration of β-Carotene and A_{Sample} is the absorbance of the remaining concentration of β-Carotene.

2.4.2. DPPH radical scavenging activity

The free radical scavenging activity of Lavander and Lavandin essential oils was determined using the method proposed by Brand-Williams et al. (1995) with slight modifications previously described by Kıvrak (2015). The extract solutions with different concentrations (40 µL) and ethanolic solution (120 µL) containing DPPH radicals (0.4 mM) were incubated at room temperature in darkness for 30 min. Absorbance was measured at 517 nm. The radical scavenging activity was calculated as a percentage of DPPH discoloration. The capability of scavenging the DPPH free radical was calculated by using Eq. (2).

$$\text{DPPH radical scavenging effect (\%)} = (A_{Control} - A_{Sample})/A_{Control} \times 100 \quad (2)$$

where $A_{Control}$ is the initial concentration of the DPPH· and A_{Sample} is the absorbance of the remaining concentration of DPPH.

2.4.3. ABTS cation radical decolorization assay

The spectrophotometric analysis of $ABTS^{\cdot+}$ scavenging activity were assessed according to the previously described method of Re et al. (1999) with some modifications (Kıvrak, 2015). The ABTS (7 mM) in water and potassium persulfate (2.45 mM) reacted to give $ABTS^{\cdot+}$, stored in the dark at room temperature for 12 h, and oxidation of ABTS appeared immediately; however, the stability of absorbance was gained after 6 h. Then, the sample solution (40 µL) in ethanol at different concentrations were mixed with $ABTS^{\cdot+}$ solution (160 µL), giving the absorbance at 734 nm by using a 96-well microplate reader in 10 min. The results were expressed by the concentration providing 50% scavenging concentration (SC_{50}). The scavenging capability of ABTS cation radical was calculated using Eq. (3).

$$\text{ABTS scavenging effect (\%)} = (A_{Control} - A_{Sample})/A_{Control} \times 100 \quad (3)$$

where $A_{Control}$ is the initial concentration of the ABTS cation radical and A_{Sample} is the absorbance of the remaining concentration of ABTS cation radical in the presence of sample.

2.5. Refractive index of essential oils

The refractive index of an essential oil is a unique number that designates how the oil responds to light and bends light. Essentially, it is a measurement that tests how the speed of light is altered when passing through the oil. Refractive index is used for quality control analysis of essential oils via comparison technique with proficiency samples. Refractive index was analyzed using standard procedures: ISO 280:1998. Essential oils were subjected to tests of index of refraction using a Mettler Toledo RM40 Refractometer (Switzerland). Calibration of the instruments was performed by measuring the ultrapure water. The samples were directly injected into the prism assembly of the instrument. An average of three measurements was taken for each sample.

2.6. Statistical analysis

The average values of each parameter were calculated by using the STATISTICA for Windows release 5.0., and reported as mean ± standard deviation. Principal Components Analysis (PCA) was performed using a Partial Least Square (PLS) Toolbox statistical package in the STATISTICA Package program (Stat-Soft, Inc., 5.0). Data were subjected to analysis of variance (ANOVA) and the LSD (least significant difference) value, to identify pairs of means that are significantly different, was calculated using the STATISTICA for Windows release 5.0.

3. Results and discussion

3.1. Essential oil analysis

The chemical compositions of sixty-six essential oils, obtained from lavander and lavandin cultivars were analyzed using GC/MS. Essential oil composition percentages of varieties are displayed in Table 1. Total ion chromatograms of essential oil of eight lavender and lavandin varieties were display in Fig. 1.

The essential oils were listed according to the International Standard ISO 3515 (2002) Oil of Lavander (*Lavandula angustifolia* Mill.) regulation, and ANOVA of the data showed that the effects of cultivars

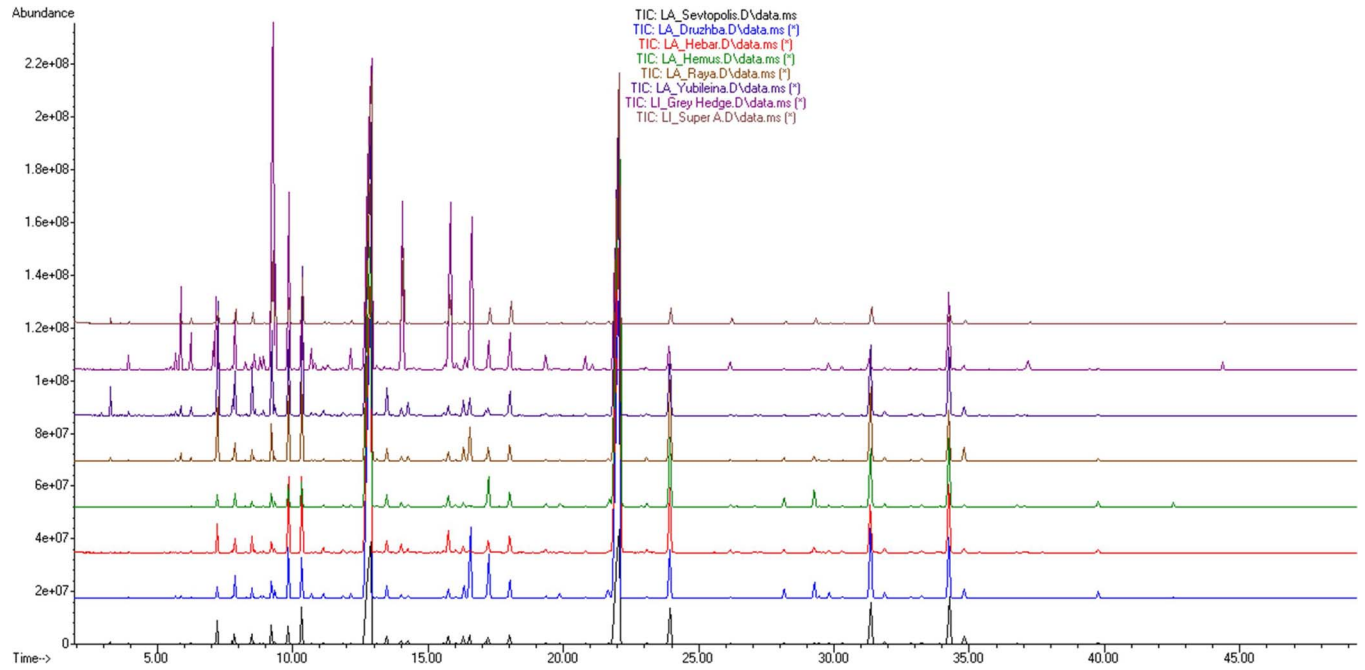

Fig. 1. GC/MS chromatograms of Lavander and Lavandin essential oil components.

Table 2
Analysis of variance (ANOVA) and least significant difference (LSD) analysis of essential oil components in *Lavandula angustifolia* cultivars ("Sevtopolis", "Yubileina", "Druzhba", "Raya", "Hebar", "Hemus") and *L. x intermedia* cultivars ("Super A", "Grey Hedge").

	Lavandula angustifolia						*Lavandula x intermedia*	
Essential oil	Sevtopolis cultivar (LAS)	Yubileina cultivar (LAY)	Druzhba cultivar (LAD)	Raya cultivar (LAR)	Hebar cultivar (LAHb)	Hemus cultivar (LAHm)	Super A cultivar (LISA)	Grey Hedge cultivar (LIGH)
α-Pinene	0.094 ± 0.003 e	0.259 ± 0.007 b	0.056 ± 0.005 f	0.200 ± 0.011 d	0.025 ± 0.010 g	0.015 ± 0,007 g	0.244 ± 0.009 c	1.527 ± 0.014 a
β-Pinene	0.084 ± 0.003 c	0.065 ± 0.013 d	0.013 ± 0.008 e	0.077 ± 0.009 c,d	0.011 ± 0.009 e	0.016 ± 0,006 e	0.147 ± 0.012 b	1.628 ± 0.003 a
3-Octanone	1.761 ± 0.004 b	4.003 ± 0.011 a	0.390 ± 0.011 f	2.043 ± 0.017c	0.878 ± 0.008 d	0.395 ± 0.007 f	0.595 ± 0.011 e	0.161 ± 0.007 g
Eucalyptol	1.640 ± 0.003d	3.029 ± 0.006 c	0.615 ± 0.013 f	1.274 ± 0.011 e	0.404 ± 0.005h	0.505 ± 0,009 g	4.051 ± 0.010 b	15.650 ± 0.007 a
α-Limonene	0.224 ± 0.002 e	0.368 ± 0.005 c	0.283 ± 0.012 d	0.162 ± 0.007 g	0.148 ± 0.007 h	0.175 ± 0.007 f	0.707 ± 0.007 b	2.349 ± 0.008 a
β-*trans*-Ocimene	1.587 ± 0.005 f	3.147 ± 0.006 b	1.742 ± 0.017 e	2.668 ± 0.007 d	2.766 ± 0.005 c	0.804 ± 0,005 h	1.452 ± 0.011 g	5.027 ± 0.007 a
β-*cis*-Ocimene	3.317 ± 0.006 b	5.929 ± 0.008 a	1.482 ± 0.014 g	3.119 ± 0.008 c	2.860 ± 0.006 d	0.916 ± 0,008 h	2.647 ± 0.013 e	1.827 ± 0.007 f
Linalool	28.102 ± 0.009 h	30.455 ± 0.011 d	35.113 ± 0.013 c	35.491 ± 0.008 b	28.782 ± 0.004 f	29.349 ± 0.008 e	36.801 ± 0.010 a	28.486 ± 0.012 g
1-octen-3-ol, acetate	0.808 ± 0.008 b	1.181 ± 0.008 a	0.582 ± 0.014 c	0.519 ± 0.008 e	0.536 ± 0.007d	0.571 ± 0.006 c	0.175 ± 0.010 f	0.008 ± 0.007 g
Camphor	0.385 ± 0.007 c	0.309 ± 0.008 d	0.185 ± 0.018 g	0.209 ± 0.008 e	0.384 ± 0.003 c	0.203 ± 0,004 e,f	5.261 ± 0.013 b	6.355 ± 0.012 a
Borneol	0.919 ± 0.008 d	0.458 ± 0.009 f	0.446 ± 0.020 f,g	0.433 ± 0.006 g	1.022 ± 0.007 c	0.529 ± 0,003 e	2.161 ± 0.011 b	8.108 ± 0.008 a
Lavandulol	0.861 ± 0.008 a	0.697 ± 0.014 b	0.656 ± 0.020 d	0.675 ± 0.009 c	0.344 ± 0.005 f	0.227 ± 0,005 g	0.145 ± 0.011 h	0.464 ± 0.011 e
Terpinen-4-ol	1.081 ± 0.010 d	0.883 ± 0.013 e	3.693 ± 0.033 b	1.661 ± 0.009 c	0.187 ± 0.010 f	0.065 ± 0,005 g	0.036 ± 0.012 h	6.880 ± 0.013 a
α-Terpineol	0.844 ± 0.008 e	0.338 ± 0.007 g	2.236 ± 0.029 a	0.635 ± 0.010 f	0.632 ± 0.007 f	1.575 ± 0.006 b	1.145 ± 0.010 c	0.998 ± 0.005 d
Linalyl acetate	32.121 ± 0.008 f	29.098 ± 0.012 g	33.297 ± 0.033 d	33.923 ± 0.007 c	45.593 ± 0.006 b	46.887 ± 0.008 a	33.087 ± 0.009 e	4.648 ± 0.012 h
Lavandulol acetate	4.840 ± 0.004 a	3.386 ± 0.008 e	2.607 ± 0.025 f	4.337 ± 0.006 b	3.416 ± 0.011 d	3.922 ± 0.006 c	1.230 ± 0.015 g	0.831 ± 0.009 h
β-Caryophyllene	6.290 ± 0.009 a	4.205 ± 0.011 d	4.784 ± 0.037 b	4.266 ± 0.008 c	2.745 ± 0.009 f	3.514 ± 0,005 e	1.545 ± 0.011 g	0.637 ± 0.011 h
β-Farnesene	7.053 ± 0.007 a	3.888 ± 0.002 c	3.497 ± 0.054 d	2.731 ± 0.007 f	3.881 ± 0.008 c	3.999 ± 0.006 b	0.914 ± 0.011 g	3.296 ± 0.010 e

a–h Different letters in each column correspond to significantly different values ($p < 0.05$).

were statistically significant ($p < 0.05$) in the content of all detected essential oil. The results of ANOVA and LSD analyses for all oils are shown in Table 2.

GC/MS analyses of essential oils showed sixty-six components including linalyl acetate (4.648–46.887%), linalool (28.102–36.801%), β-farnesene (0.914–7.053%), β-caryophyllene (0.637–6.290%), lavandulol acetate (0.831–4.840%) as the main components. Linalool and linalyl acetate were predominant compound in studied lavender and lavandin cultivars in this work.

The highest linalyl acetate content was found in *L. angustifolia* Hemus (LAHm) (46.887%) while the highest linalool content was found in *Lavandula x intermedia* Super A (LISA) (36.801%). The data are in accordance with International Standard ISO 3515 (2002) Oil of Lavander (*Lavandula angustifolia* Mill.).

Previous studies showed that the ranges of linalool and linalyl acetate in essential oil of *L. angustifolia* are respectively 10.0–57.5% and 4–55% (Smigielski et al., 2009). According to the Raina and Negi, (2012), linalool and linalyl acetate contents of lavender oil are used as the criterion of quality that has a high demand in the international markets.

One of the main reasons of this demand can be explained by the report on sedative effects of lavender oil caused by the major components linalyl acetate and β-linalool. These compounds can be rapidly absorbed through the body by inhalation with plasma level reaching a maximum peak in approximately seven minutes after administration, which can cause a depression of nervous system. Linalyl acetate has a narcotic action and linalool acts as a sedative (Sayorwan et al., 2012).

Another reason of this demand is common applications of linalyl acetate in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other non-toiletries as well as non-cosmetic products, household cleaners, and detergents as fragrant component. Production volume per year of this compound is 1000 metric ton. Besides, linalool is used in flavor and fragrances (in the form of its esters) and has a production volume of 2.0–2.2 million metric tons (Kiran Babu et al., 2016). Gliszczyńska et al. (2011) reported that (R)-Lavandulol and its esters have been identified in the pheromones of some global insect pests.

Additionally, *L. angustifolia* Sevtopolis displayed highest lavandulol acetate content as 4.840%, and Raya (4.337%), Hemus (3.922%), Hebar (3.416%), Yubileina (3.386%) and Druzhba (2.607%) cultures, respectively. Other significant component β-caryophllene was indicated highest in *L. angustifolia* Sevtopolis (LAS) (6.290%). Caryophyllene has a wide application area, for example, as odor and taste-modifying agents, potential anticarcinogenic agents, the control of whitefly species, a repellent for pine wood nematodes and as a component of antitumor compositions. Caryophyllenes, more particularly alpha humulene or beta caryophyllene as we identified in essential oils of cultivars of *L. angustifolia*, are used as an anti-inflammatory and as an analgesic according to Maimulyanti and Prihadi, (2016).

There is a limitation for camphor content as lower than 0.5% in International Organization for Standardization (ISO 3515:2002). The studied samples of *L. angustifolia* var. satisfied the respected range (0.185–0.385%). However; *L. x intermedia* Super A and *L. x intermedia* Grey Hedge varieties possessed camphor content higher than 5%. Linalyl acetate content of all studied samples of lavender and lavandin except *L. x intermedia* Grey Hedge (4.648%) provided the requirement of the standard as 29.098–46.887%. All results were obtained by Zagorcheva et al. (2013) and Stanev et al. (2016) for these cultivars are close to the results determined in this present study. The obtained results are also in agreement with other studies performed for essential oils isolated from different subspecies and cultivars of *L. angustifolia* (Kara and Baydar, 2013; Renaud et al., 2001).

These six cultivars of *L. angustifolia* var. were approximately suitable for lavender oil standard (ISO 3515:2002) because the linalyl acetate content was higher than linalool content, and had low camphor content in all crop.

Standard 3515:2002 clarifies acceptable ranges for the major

Table 3
Antioxidant activity of Lavandin and Lavander species by β-carotene bleaching, ABTS· and DPPH·assays.

Essential Oils and Standards	Antioxidant Activity		
	β-Carotene-linoleic acid assay IC_{50} (μg mL^{-1})	ABTS·$^{+}$ assay SC_{50} (μg mL^{-1})	DPPH· assay SC_{50} (μg mL^{-1})
L. angustifolia Sevtopolis	24.28 ± 0.10	61.23 ± 0.11	91.56 ± 0.08
L. angustifolia Yubileina	23.67 ± 0.14	71.50 ± 0.10	102.34 ± 0.14
L. angustifolia Druzhba	23.71 ± 0.09	74.08 ± 0.13	97.61 ± 0.15
L. angustifolia Raya	27.09 ± 0.12	67.39 ± 0.10	105.08 ± 0.08
L. angustifolia Hebar	24.46 ± 0.10	65.00 ± 0.12	99.60 ± 0.12
L. angustifolia Hemus	24.68 ± 0.16	67.50 ± 0.10	101.27 ± 0.10
L. x intermedia Super A	30.04 ± 0.10	61.64 ± 0.07	89.81 ± 0.17
L. x intermedia Grey Hedge	39.52 ± 0.20	64.32 ± 0.10	93.20 ± 0.10
α-Tocopherol	5.57 ± 0.10	11.90 ± 0.10	16.48 ± 0.12

IC_{50}: 50% inhibition concentration; SC_{50}:50% scavenging concentration;
Values represent the means ± SD of three parallel measurements ($p < 0.05$).

components of *L. angustifolia* essential oil: 1,8-cineole, 0–3%; limonene, 0–1%; trans-β-ocimene, 0.5–6%; *cis*-β-ocimene, 1–10%; 3-octanone, 0–3%; camphor, 0–1.5%; linalool, 20–43%; linalyl acetate, 25–47%; terpinen-4-ol, 0–8%; lavandulol, 0,3–3%; lavandulol acetate, 1–8%; α-terpineol, 0–2%.

It is considerated that among the all lavender cultivars, *L. angustifolia* Hemus (LAHm) and *L. angustifolia* Hebar (LAHb) could be identified as quality cultivars according to ISO 3515:2002 lavender oil standards owing to high contents of linalyl acetate, linalool and low contents of camphor. Such variability depends on several factors including local climatic and environmental conditions, season, geographical location, geology, genetic/chemotypic, nutritional status of the plants, part of the plant used and isolation process according to Gharib et al. (2013).

The studied *L. angustifolia* and *L. x intermedia* cultivars revealed good antioxidant activities. Table 3 shows the antioxidant activity of the different cultivars of lavandin and lavander for β-carotene/linoleic acid bleaching assay (lipid peroxidation inhibition), DPPH· and ABTS ·$^{+}$ assays (radical scavenging activity) compared with α-tocopherol.

The essential oils of *lavandula* var., obtained via vapor distillation technique, firstly were dissolved in ethyl alcohol and then solutions of 5000 μg mL^{-1} in concentrations for each sample. Their activities were measured. In the β-Carotene-linoleic acid assays, the highest inhibition was found in *L. angustifolia* Yubileina (23.67 ± 0.14 μg mL^{-1}). The other lavandin and lavender var. also presented similar activities. In the ABTS cation radical and DPPH free radical scavenging assays showed that *L. angustifolia* Sevtopolis (61.23 ± 0.11 μg mL^{-1}) and *L. x intermedia* Super A (89.81 ± 0.17 μg mL^{-1}) were evaluated the highest activities, respectively. The studied samples displayed resembling results, those were comparable with the standard α-tocopherol. In the ABTS·$^{+}$ assay, the *L. angustifolia* Druzhba (74.08 ± 0.13 μg mL^{-1}) cultivar exhibited lowest radical scavenging activity.

3.2. Refractive index composition

In this study refractive index values of lavender and lavandin cultures samples were examined at 20 °C using digital refractometer (Mettler Toledo RM40 Refractometer, Switzerland). In ISO 3515:2002 (E) regulation for refractive index analysis, limit values are between 1.4590–1.4630 Bulgaria principal origin lavenders, 1.4600–1.4660 other principal origin lavenders. Grey Hedge (LIGH) cultivar belonging to *L. x intermedia* var. had 1.4652 refractive index which is in the acceptable range of the standard. Refractive index values of *L. angustifolia* var. Bulgaria cultivar samples (LAS, LAY, LAD, LAR, LAHb, LAHm) were in the range of 1.4582–1.4629.

3.3. Principal component analysis

Principal component analysis (PCA) is one of the main approaches in chemometrics, and it is widely used for the classification study in the field of food research (Yu, 2005). PCA is a statistical data reduction method. It transforms the original set of variables to a new set of uncorrelated variables called principal components (PCs). By plotting the PCA scores, it is possible to visually assess similarities between samples and determine whether samples can be grouped (Kıvrak and Kıvrak, 2017). PCA was generated three significant principal components (PCs) explaining 51.89, 21.24 and 11.32% of the total variance respectively. Fig. 2 is a plot of principal component loadings of quality properties in terms of essential oil on the first and second principal components, PC1, PC2. PC1 explained mainly variation in lavandulol, lavandulol acetate, β-farnesene, β-caryophyllene, 1-octen-3-ol acetate, 3-octanone and β-*cis*-ocimene which were in positive area. Linalool and linalyl acetate were in positive area for two of the principal components, PC1, PC2. Moreover, PC2 displayed variation in camphor and α-terpineol that were in positive side. Eucalyptol, α-pinene, β-pinene, α-limonene, borneol, terpinen-4-ol and β-*cis*-ocimene exhibited in negative side for two of the principal components, PC1, PC2.

The loadings indicated that lavender quality increases as the value of PC1 became increasingly positive for higher values of linalyl acetate than linalool as specified in the standard.

In PCA loadings of quality properties, PC1 explained mainly variation in camphor which was in negative area (Fig. 2). As it is to provide complementary information to the factor analysis, *L. x intermedia* var. Super A and Grey Hedge cultures (LISA and LIGH) are in negative PC1. On the other hand, *L. angustifolia* var. (LAS, LAY, LAD, LAR, LAHb, LAHm) cultivars positioned on positive PC1 (Fig. 3). *L. angustifolia* var. (LAD, LAHb, LAHm) cultivars were displayed in the positive side of PC1 and PC2. This finding overlapped with higher linalyl acetate and linalool contents as those were positioned in Fig. 2. The factor analyses provide complementary information to search for grouping among lavender and lavandin cultures. When Fig. 3 is examined, findings of PC1 indicated the difference between *L. angustifolia* and *L. x intermedia* varieties. Nevertheless, *L. angustifolia* var. were in the positive side of PC1, and *L. x intermedia* var. were in the negative side of PC1.

4. Conclusion

This present survey illuminated a detailed qualification of the eighteen essential oil components of the eight lavender and lavandin cultures samples belonging to *L. angustifola* and *L. x intermedia* cultivars commercially grown in Turkey, those are rich in linalyl acetate, linalool and poor in camphor, those are compatible with the legislation of international standard. Also the studied lavender and lavandin samples have high antioxidant activity.

L. x intermedia Super A cultivar had been preferred to be grown by the producers in western Anatolia. Recently, cultivation of *L. angustifolia* Sevtopolis was increasingly grown by the producers in that region. This raise is due to low camphor content of *L. angustifolia* varieties,

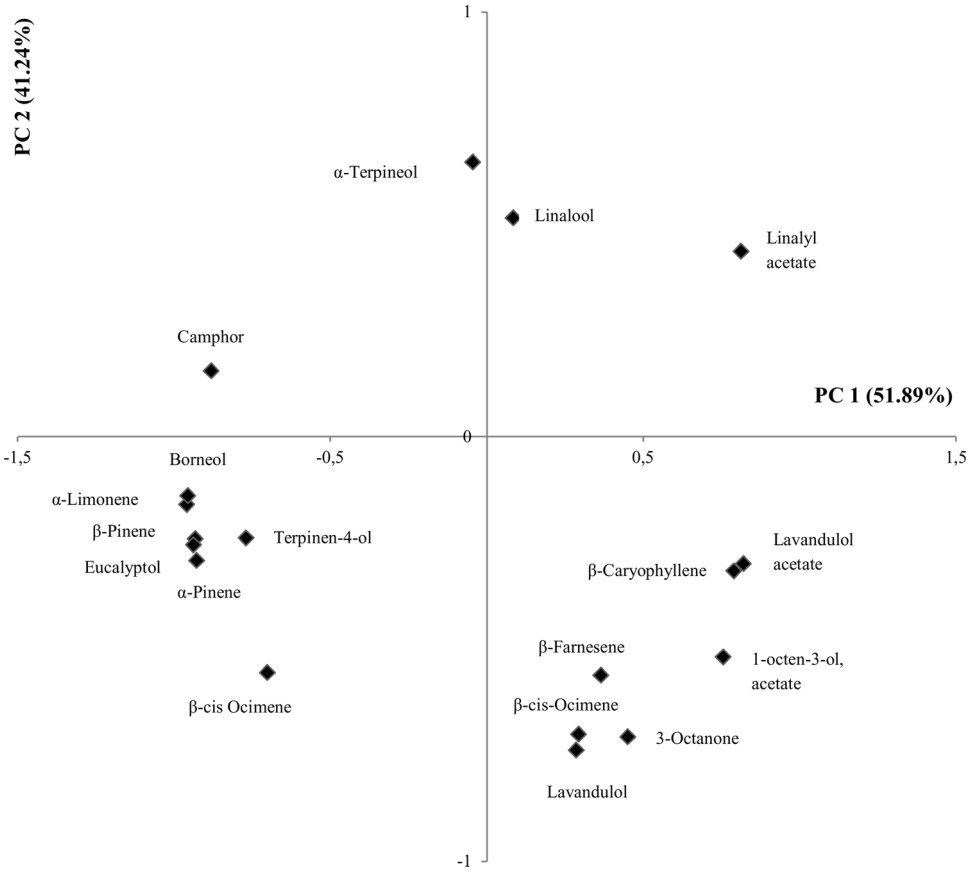

Fig. 2. Loading plot of first and second principal components from 18 different components in the list of International Standard ISO 3515 (2002).

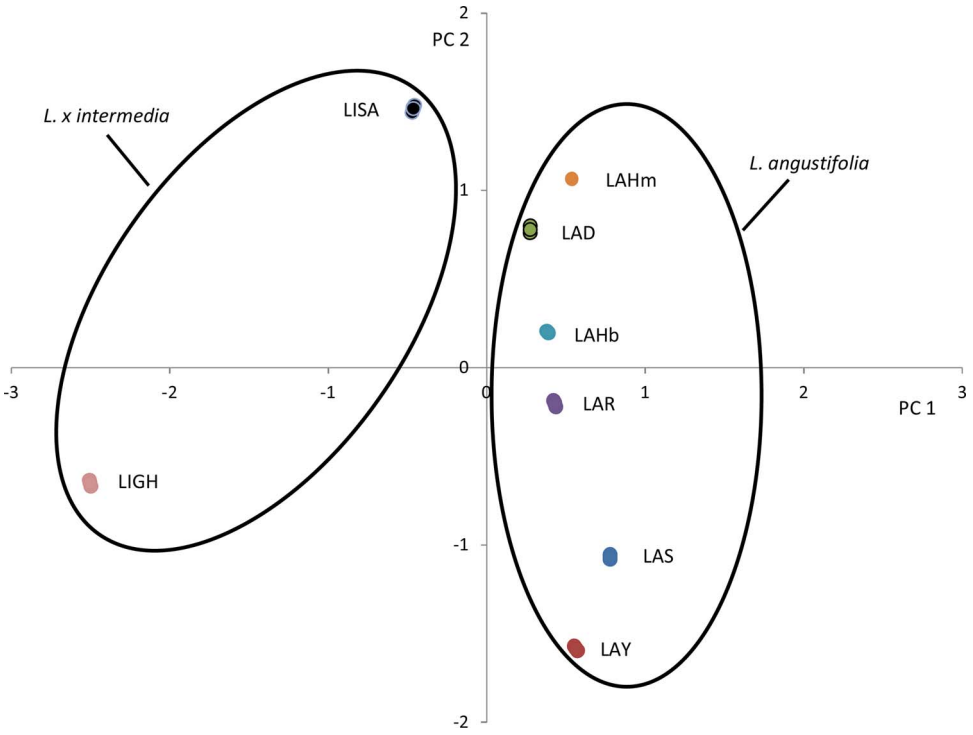

Fig. 3. Principal component analysis (PCA) of laverder and lavandin samples, *L. angustifolia* Mill. LAS: Sevtopolis, LAY: Yubileina, LAD: Druzhba, LAR: Raya, LAHb: Hebar, LAHm: Hemus and *L. x intermedia* LISA: Super A, LIGH: Grey Hedge.

which have below < 0.5% comparing to *L. x intermedia* Super A cultivar. Further, cultivation of *L. angustifolia* varieties increases rapidly in western Anatolia.

All cultivars may be considered as a natural raw material source for pharmaceuticals and cosmetic products. These results verified industrial usage of these plants. It is suggested that essential oil ingredients of *L. angustifolia* and *L. x intermedia* cultivars make those worth to cultivate.

Conflict of interest

There is no conflict of interest.

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