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Research Article

General characteristics of lavender biomass (*Lavandula angustifolia* Mill.) before and after industrial distillation

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Abstract

The aim of the present study was to evaluate and compare three types of lavender *(Lavandula angustifolia* Mill.*)* biomasses for their content of biologically active substances and potential application. The first one was from unprocessed lavender (L-UNTR22_Z) and the other two were industrial steam-distilled lavender solid residues (L-SD22_Z and L-SD22_M). Ash, protein, polyuronide content and the degree of esterification of the pectic substances in the biomass were investigated. 70% ethanolic extracts of the residues were prepared and their total polyphenol content and antioxidant activity were evaluated by four methods. Individual phenolic acids, flavonoids, polar non-volatile compounds and polar volatile compounds were also determined by chromatographic analyses. The highest degree of esterification (83.9±0.5%) and protein content (8.15±1.6%) were found in L-SD22_Z extract. L-SD22_M extract demonstrated the highest polyphenolic content and antioxidant activity. Six phenolic acids (protocatehuic, syringic, *p*-coumaric, ferulic, salicylic, and gallic acid) and five flavonoids ((+)-catechin, (-)-epicatechin, rutin, hesperidin, and quercetin) were detected by HPLC analyses. The detail information about amino acids, sugars, organic and phenolic acids, as well as polar volatile compounds was obtained by GC-MS. The results suggested that the lavender (untreated and residual biomass) are a good source of dietary antioxidants and compounds with potent biological activity.

Keywords

Lavandula angustifolia Mill., biomass valorization, polyphenols, antioxidant activity, ethanol-water extract

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Introduction

Lavender (*Lavandula angustifolia* Mill.) is a perennial herbaceous plant of the Lamiaceae family (Labiatae), which originates from the Mediterranean region and is cultivated worldwide. Lavender essential oil is widely used in the cosmetic industry and for the preparation of goods including fragrances, colognes, skin lotions, soaps, food flavorings, perfumes and aromatherapy drugs (Tabata et al. 2015). Lavender essential oil is mainly produced from glands on the surface of flowers and leaves. The genus Lavandula is divided into 37 species depending on the shape of the leaves, the corolla morphology, sepals and bracts (Upson et al. 2002). Only the essential oils of three lavender species (*Lavandula angustifolia*, *Lavandula latifolia* and *Lavandula* hybrid) are regarded as important for the perfumery and cosmetics industry (Dong et al. 2020).

It is a well-known fact that the main reason for the huge amounts of solid residues generated during the industrial processing of lavender are the small amounts of essential oil in the fresh plant material (*L. angustifolia* – 0.8-1.3%) (Marovska et al. 2022a; 2022b; Slavov et al. 2018). The lavender residual biomass is usually discarded as unnecessary in places near the processing facilities. Although biodegradable, the residues pose a real risk to the ecological balance of the ecosystems. The practice of discarding solid lavender and rose liquid residues, in turn, led to the fact that some of the largest distilleries in Bulgaria were forced to pay fines. However, the residues can be used not only for the preparation of biocomposites in construction, but also for insulation materials, and cartons (Angelova et al. 2021). The extracts from industrial lavender solid residues were successfully applied for "green" synthesis of metal nanoparticles with potential application in electrochemistry (Lazarova et al. 2019). Additionally, other valuable substances, such as polysaccharides, antioxidants, and aroma substances could be obtained (Marovska et al. 2022a; 2022b). To the best of our knowledge, the information about the composition, chemical properties and antioxidant activity of lavender post-distillation residues is scarce. Therefore, the aim of the present study was evaluation of the chemical composition of lavender residual biomass.

Materials and Methods

The lavender solid residue (L-SD22_Z) and the untreated lavender biomass (L-UNTR22_Z) were provided by GALEN–N distillery (Zelenikovo, Bulgaria, 2022). The second solid residual biomass (L-SD22_M) was obtained from ECOMAAT distillery (Mirkovo, Bulgaria, 2022). Both solid residues resulted from industrial steam distillation of fresh lavender stalks and flowers. Unwanted impurities were carefully removed. The biomass was air-dried before subjected to analyses.

All the reagents used were of analytical grade and were purchased from local distributors.

Water- ethanolic extracts of the solid residues were prepared as follows: The crude material (300 g) was weighed and transferred to a 5000 mL flask containing 2000 mL of 70% (v/v) ethanol preheated in a water bath to 60-65°C. The temperature was maintained for 1 hour, after which the mixture was allowed to stand overnight at room temperature. The mixture was filtered through a nylon cloth and a new portion of solvent (1000 mL 70% ethanol) was added to the solid residue, heated to 60-65°C, left for 1 h in a water bath with continuous stirring, and filtered again. After the final filtration, the filtrates were combined and used for further analyses (Marovska et al. 2023).

The ash content was estimated after igniting the samples at 605°C in a muffle furnace (MLW 212.11, MLW, Germany) to a constant weight. The determination of the protein content was performed according to the AOAC Method 976.06 using automated Kjeldahl system MultiKjel K-365 with potentiometric titrator Metrohm ECO (Büchi Labortechnik, Switzerland) and conversion factor 6.25 for converting the amount of nitrogen to protein.

The polyuronide content (PUC) and the degree of esterification (DE) of the pectic substances in the plant biomass was determined according to (Owens et al. 1952): The lavender samples (1 g) were weighted and mixed with 5 mL 95% ethanol, 100 mL deionized water and 1 g NaCl. The mass was stirred mechanically for 15 min on a magnetic stirrer and 6 drops of Hinton's reagent (mixture of 20 mL 0.4% Bromothymol blue, 60 mL 0.4% Phenol Red, 20 mL 0.4% Cresol Red ethanol solutions, and 20 mL distilled water) were added. The sample was

titrated with 0.1 N NaOH until the color change retain for 1 min. Then 40 mL 0.1 N NaOH were added and allowed to stay 120 min at room temperature. To the solution 50 mL 0.1 N H_2SO_4 were added and second titration with 0.1 N NaOH was performed. A blank was prepared using deionized water instead of sample solution. The degree of esterification and polyuronide content were calculated using the equations:

$$\text{DE}, \% = \frac{\text{B}}{\text{A+B}} \times 100, \quad (1)$$

$$\text{PUC}, \% = \frac{\text{F} \times (\text{A} \times 1.76 + \text{B} \times 1.9)}{\text{m}}, \quad (2)$$

where $\text{A} = a - a_1$, and $\text{B} = b - b_1$

a - first titration, mL

a_1 - first titration blank, mL

b - second titration, mL

b_1 - second titration blank, mL

m - sample mass, g

F - factor of 0.1N NaOH

The Folin-Ciocalteu method was used to determine total phenolic content according to (Nurcholis et al. 2021) with slight modifications. Briefly, 0.2 mL of the extracts were mixed with 1 mL of five-times diluted aqueous Folin-Ciocalteu solution, stirred and left for 20 min. Then, 0.8 mL 7.5% Na_2CO_3 solution was added. The mixture was further incubated at 25℃for 20 min and the absorbance was measured at 765 nm using Camspec M107 UV-Vis spectrophotometer (Camspec Ltd., Cambridge, UK) against the blank. Gallic acid (GA) was employed as a standard and results were presented as mg GA equivalents. The total flavonoid content was analyzed using 10% $Al(NO_3)_3$ as described by (Popova et al. 2022).

The antioxidant activity of 70% ethanolic extracts was determined by four methods: 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging ability, the radical 2,2′-azinobis (3-ethylbenzothiazoline-6-sulphonic acid – $ABTS^{\bullet+}$) assay, ferric reducing antioxidant power (FRAP) assay and cupric ion reducing antioxidant capacity (CUPRAC) as described in details by (Marovska et al. 2023). HPLC analyses for determination of individual phenolic acids and flavonoids were performed according to (Vrancheva et al. 2021). Chromatographic separation and determination of individual phenolic acids and flavonoids was performed on a Hitachi LaChrom Elite® HPLC system (Hitachi High Technologies America, Inc., Schaumburg, Illinois, USA), coupled with diode-array detector (DAD, L-2455) and EZChrom Elite™ software.

The individual volatile and non-volatile compounds in the 70% ethanolic extracts were determined by GC-MS analyses. It was carried out on a 7890A gas chromatograph, interfaced with a mass selective detector 5975C Agilent Technology 5975C inert XL EI/CI MSD (Agilent, USA) (Marovska et al. 2023).

Assays were performed in triplicate and data are presented as mean values. Statistical significance was detected by analysis of variance (ANOVA; p value<0.05 indicates statistical difference).

Results

Collection, inspection and drying of the starting raw material. The collection and processing of the lavender stalks and stems has a campaign character, and in addition essential oil plants (like all biological objects) differ in their chemical composition within certain ranges from year to year. This is also valid for the unprocessed lavender and the residues from the industrial steam distillation. Hence, the idea was to collect materials from untreated and industrially processed (steam-distilled) lavender from two distilleries (located in different regions and collected in July-August 2022) and subsequently make a comparison, including also data from previous years (Marovska et al. 2022b; Slavov et al. 2018). With this approach, the aim was obtaining additional information on the composition of a given raw material over more than one year and from different locations. Once the steam distillation of the raw lavender had been completed and the material was removed from the distillation still, the solid residues were cooled and inspected to remove unwanted impurities (insects, minerals, weeds, etc.).

General characteristics of the lavender biomass. Comparing the results for the two types of lavender residues, L-SD22_M and L-SD22_Z and unprocessed lavender L-UNTR22_Z, it could be concluded that the ash content is the highest in the L-SD22_Z (7.45±0.20%) (Table 1). The same sample was characterized also with the highest polyuronide content: 8.0±0.2%.

Table 1. General characteristics of the lavender biomass

№	Lavender sample	Protein, %	Ash, %	PUC, %	DE, %
1	L-SD22_M	8.15±1.60[a]	5.41±0.07[b]	7.5±0.1[b]	83.9±0.5[a]
2	L-SD22_Z	6.72±0.28[b]	7.45±0.20[a]	8.0±0.2[a]	78.9±0.5[c]
3	L-UNTR22_Z	5.94±0.12[c]	4.10±0.30[c]	7.1±0.1[c]	81.6±0.3[b]

The results are presented as an average of three determinations ± SD;
DE – degree of esterification of the pectic substances; PUC – polyuronide content
[a,b,c] values with different letters in a column are significantly different (one way ANOVA, $p < 0.05$

The sample L-SD22_M had the highest DE (83.9±0.5%) and protein content (8.15±1.6%).

Total phenolics, flavonoids and antioxidant activity of lavender 70% ethanol extracts. The initial raw materials were subjected to extraction with hot 70% ethanol. This pretreatment lead to extraction of low-molecular substancesgfdhd (phenolic compounds, sugars, pigments, etc.) and obtaining of extracts which could serve as a potential source of dietary antioxidants. Hence, the resulting 70% ethanol extracts were analyzed for their total phenolics, flavonoids content and antioxidant properties. The results of the analysis are presented in Table 2 and the correlation between the total phenolics content and antioxidant activity is shown in Table 3.

The highest polyphenol content (13.85±0.26 mg GAE.g^{-1}) exhibited the L-SD22_M sample, while L-UNTR22_Z was the richest in total flavonoids (1.23±0.04). Usually, part of the flavonoids could be destroyed due to the elevated temperature during the steam distillation and this could be the reason for the higher flavonoid content in L-UNTR22_Z. Nevertheless, the highest antioxidant activity was shown by the two lavender residues with L-SD22_M showing slightly higher results for the antioxidant capacity measured by all four methods. These results suggested that mostly the polyphenolic compounds were the responsible for the observed higher antioxidant activity in the processed samples. Comparing the results for the total polyphenolic content in the residual biomass (2022 crop; present study) with two residues from 2016 crop (one steam distilled and one extracted with supercritical CO_2 lavender; (Slavov et al. 2018) and three residues from 2020 and 2021 crops (Marovska et al. 2023) it could be concluded that the amount of polyphenols found in the residual biomasses is similar and range from 4.45±0.17 to 16.08±0.38 mg AE.g^{-1} lavender residue. Total flavonoids content, however, was 2-3 times lower than the residues from the previous years' crops. These observations might be mainly due to the initial content of polyphenolic compounds and flavonoids in the fresh lavender biomass, as this could be observed confronting the values for total polyphenolics in L-UNTR22_Z: 4.45±0.17 mg GAE.g^{-1} (present study) and L-UNTR21 Z: 15.02±0.22 mg GAE.g^{-1} (Marovska et al. 2023). Additionally, the extent of steam distillation, the removal of other substances from the plant material, and the disruption of the plant cell walls (facilitating further extraction) could also play significant role in the amount of polyphenols and flavonoids in the 70% ethanol extracts.

The correlation between total antioxidant activity obtained by four methods and total phenolic and flavonoids contents were presented in Table 3. The results showed positive linear correlations between total antioxidant activities and total phenolic contents. The highest correlation was observed between total phenolics and DPPH and FRAP assays (coefficient of correlation r^2 = 0.9942 and 0.9975 for DPPH and FRAP values, respectively) (Table 3). Negative correlation exists between antioxidant capacity and total flavonoids. These results suggested that the total phenolic compounds contributed significantly to the antioxidant activity of the investigated lavender samples. In addition, the antioxidant components in the analyzed lavender samples could reduce ferric ions and scavenge free radicals. This observation about the dependence of antioxidant potential form total phenolic content were reported by other authors for different plant extracts (Li et al. 2014; Petrova et al. 2016).

Table 2. Polyphenolic content and antioxidant activity of 70% ethanol extract

Sample	Total phenolics		Total flavonoids		DPPH		ABTS		FRAP		CUPRAC	
	mg GAE.g^{-1}	mg GAE.mL^{-1}	mg QE.g^{-1}	mg QE.mL^{-1}	mM TE.g^{-1}	mM TE.mL^{-1}	mM TE.g^{-1}	mM TE.mL^{-1}	mM TE.g^{-1}	mM TE.mL^{-1}	mM TE.g^{-1}	mM TE.mL^{-1}
L-UNTR22_Z	4.45±0.17^{c}	0.57±0.02^{b}	1.23±0.04^{a}	0.16±0.01^{a}	23.49±2.17^{c}	3.61±0.33^{c}	9.95±0.21^{b}	1.53±0.03^{b}	16.93±2.21^{c}	2.61±0.34^{b}	31.93±0.24^{c}	4.91±0.04^{c}
L-SD22_Z	11.89±1.65^{b}	1.83±0.25^{a}	1.13±0.05^{b}	0.17±0.01^{a}	48.85±0.91^{b}	7.51±0.14^{b}	30.58±0.24^{a}	4.70±0.03^{a}	74.85±4.88^{b}	11.52±0.75^{a}	160.43±7.17^{b}	24.68±1.10^{b}
L-SD22_M	13.85±0.26^{a}	2.13±0.04^{a}	1.16±0.02^{a,b}	0.18±0.03^{a}	51.70±0.40^{a}	7.95±0.06^{a}	31.60±0.70^{a}	4.86±0.10^{a}	84.27±2.76^{a}	12.97±3.50^{a}	385.27±4.78^{a}	59.27±0.74^{a}

The results are presented as an average of three replications ± SD;
^{a,b,c} values with different letters in a column are significantly different (one way ANOVA, $p < 0.05$)
mg GAE – milligrams gallic acid equivalents;
mg QE – milligrams quercetin equivalents;
mM TE – millimoles Trolox equivalents.

Table 3. Correlation between total polyphenols and antioxidant activity of 70% ethanol extracts

	DPPH mg GAE.g^{-1}	ABTS mg GAE.g^{-1}	FRAP mg GAE.g^{-1}	CUPRAC mg GAE.g^{-1}
Total phenolics	0.9942	0.9875	0.9975	0.8870
Total flavonoids	-0.9039	-0.9243	-0.8872	-0.5151

*mg GAE – milligrams gallic acid equivalent

Determination of the individual phenolic acids and flavonoids in the 70 % ethanol extracts. The extracts were further examined by HPLC to determine the individual phenolic acid and flavonoids. As shown in Table 4, seven phenolic acids and five flavonoids were tentatively detected.

Table 4. Phenolic acids and flavonoids in the lavender 70% ethanolic extracts

Phenolic compounds	L-UNTR22_Z	L-SD22_Z	L-SD22_M
	Concentration, μg.mL^{-1}		
Phenolic acids			
Gallic acid	nd*	0.57±0.07	nd
Protocatehuic acid	1.47±0.09^{b}	18.91±0.16^{a}	nd
Syringic acid	43.14±0.32^{a}	5.01±0.41^{b}	3.38±0.37^{c}
p-Coumaric acid	ULOQ**	16.82±0.18^{a}	2.86±0.16^{b}
Ferulic acid	70.48±0.47^{c}	298.06±0.59^{a}	196.94±0.61^{b}
Salicylic acid	43.14±0.51^{b}	84.87±0.62^{a}	34.83±0.70^{c}
Rosmarinic acid	95.31±0.48^{c}	194.55±0.36^{a}	156.48±0.53^{b}
Flavonoids			
(+)-Catechin	nd	7.93±0.57^{b}	17.90±0.63^{a}
(-)-Epicatechin	723.29±1.98^{b}	1258.14±2.57^{a}	699.91±3.64^{c}
Rutin	84.11±0.86^{c}	208.79±0.72^{a}	176.66±0.88^{b}
Hesperidin	21.05±0.46^{b}	3.01±0.48^{c}	52.76±0.53^{a}
Quercetin	2.49±0.21^{b}	4.14±0.16^{a}	2.96±0.19^{b}

*nd – Not determined;
**ULOQ – under limit of quantification;
^{a,b,c} different letters in a row indicate significant differences (one way ANOVA, $p < 0.05$)

Ferulic acid (298.06±0.59 μg.mL^{-1}), rosmarinic acid (194.55±0.36) and salicylic acid (84.87±0.62 μg.mL^{-1}) were the dominant phenolic acids and they were found in the highest concentrations in L-SD22_Z sample. Epicatechin (1258.14±2.57 μg.mL^{-1}) and rutin (208.79±0.72 μg.mL^{-1}) were the representatives of the flavonoids present in the highest concentrations in the same extract (Table 4). Comparing the values for the individual phenolic acids content (present study) with the results for samples from 2020 and 2021 (Marovska et al. 2023) it could be concluded that the same three phenolic acids were found in the highest concentrations. Ferulic acid was predominating in the residues from 2022 harvest while rosmarinic acid was found in the highest amounts in the lavender samples from 2020 and 2021 crop.

Considering the unprocessed lavender biomass (L-UNTR22_Z), the phenolic acids in the highest amounts in the extract were rosmarinic acid (95.31±0.48 μg.mL^{-1}), ferulic acid (70.48±0.47 μg.mL^{-1} and syringic acid (43.14±0.32 μg.mL^{-1}). From the flavonoids (-)-epicatechin was found in the highest amounts: 723.29±1.98 μg.mL^{-1}.

Additional observation comparing the results for the processed and un-processed samples from the present study and the data reported by (Marovska et al. 2023) is that the amounts of most of the phenolic acids in the lavender residue are higher than in the untreated lavender biomass. This might be related to the elevated temperatures during steam distillation, which could release the phenolic compounds from their bound form (most probably present as esters or glycosides in the plant matrix). For the flavonoids these conclusions cannot be drawn since the concentrations differs in the residues and in the un-processed lavender, i.e. for (-)-epicatechin the content is lower in un-processed sample, while for hesperidin and quercetin is in opposite.

Determination of polar non-volatile and volatile (aroma) compounds in the 70% ethanol extracts. In the subsequent analyses by GC-MS, the profile of volatile and non-volatile polar compounds in the 70% ethanol extracts were determined and the results are presented in Tables 5 and 6.

Table 5. Polar non-volatile compounds present in 70% ethanolic extracts

№	Compound, mg.g^{-1} DW	RI	L-UNTR22_Z	L-SD22_Z	L-SD22_M
	Amino acids				
1	L-Valine*	1228	1.06±0.11^{a}	0.42±0.05^{c}	0.76±0.07^{b}
2	Norvaline	1244	0.41±0.12^{b}	0.58±0.03^{b}	0.96±0.05^{a}
3	L-Leucine*	1272	1.07±0.13^{a}	1.18±0.06^{a}	0.60±0.04^{b}
4	L-Isoleucine*	1298	1.48±0.10^{b}	0.51±0.02^{c}	1.78±0.13^{a}
5	L-Proline	1305	2.92±0.16^{a}	2.72±0.08^{a,b}	2.56±0.16^{b}
6	Glycine	1308	2.08±0.12^{a,b}	2.28±0.10^{a}	1.91±0.14^{b}
7	L-Serine	1362	1.90±0.11^{a}	0.23±0.02^{b}	0.17±0.01^{b}
8	L-Threonine*	1390	1.40±0.09^{b}	2.14±0.10^{a}	0.84±0.09^{c}
9	L-Aspartic acid	1508	1.16±0.08^{a}	0.35±0.01^{c}	0.83±0.04^{b}
10	L-Phenylalanine*	1646	0.58±0.06^{a}	0.59±0.03^{a}	0.66±0.05^{a}
11	L-Lysine*	1736	0.35±0.01^{c}	0.68±0.03^{a}	0.53±0.05^{b}
	Organic acids				
1	Benzoic acid	1248	1.63±0.21^{a}	0.89±0.05^{c}	1.12±0.09^{b}
2	Fumaric acid	1355	1.54±0.09^{a}	0.42±0.04^{b}	0.29±0.02^{c}
3	Malic acid	1488	0.80±0.05^{a}	0.72±0.05^{a}	0.48±0.04^{b}
4	Pyroglutamic acid	1512	0.56±0.01^{b}	1.12±0.08^{a}	1.34±0.10^{a}
5	L-Threonic acid	1528	0.40±0.03^{b}	0.27±0.02^{c}	0.94±0.03^{a}
6	Quinic acid	1842	33.28±0.19^{b}	47.50±0.12^{a}	26.11±0.15^{c}
7	Gluconic acid	1990	10.66±0.19^{c}	20.13±0.21^{b}	20.77±0.12^{a}
8	Glucaric acid	2012	0.57±0.04^{b}	0.97±0.06^{a}	0.34±0.05^{c}
9	Succinic acid	1311	5.86±0.18^{a}	3.14±0.11^{c}	3.94±0.17^{b}
10	Cinnamic acid	1547	1.06±0.06^{b}	1.44±0.07^{a}	0.67±0.02^{c}
11	Glutamic acid	1609	0.79±0.04^{b}	0.42±0.05^{c}	2.81±0.10^{a}
12	p-Coumaric acid	1945	3.13±0.10^{c}	5.00±0.13^{a}	4.39±0.18^{b}
13	Ferulic acid	2101	3.64±0.11^{b}	4.24±0.10^{a}	3.79±0.12^{b}
	Phenolic acids				
1	Salycilic acid	1516	1.03±0.06^{a}	0.43±0.03^{c}	0.76±0.03^{b}
2	Vanillic acid	1757	0.27±0.03^{b}	0.37±0.02^{a}	0.18±0.02^{c}
3	Protocatechuic acid	1813	0.43±0.05^{c}	0.62±0.04^{b}	0.80±0.08^{a}
4	Syringic acid	1888	0.65±0.08^{b}	0.83±0.05^{a,b}	0.93±0.10^{a}
5	Caffeic acid	2139	2.33±0.14^{c}	7.37±0.12^{a}	5.76±0.09^{b}
6	Galactonic acid	1996	5.91±0.11^{a}	5.12±0.10^{b}	3.04±0.09^{c}

Table 5. Polar non-volatile compounds present in 70% ethanolic extracts - Continuation

	Sugars				
1	Xylose isomer	1633	1.67±0.10[a]	0.90±0.08[c]	1.21±0.09[b]
2	Xylose isomer	1642	1.24±0.08[a]	0.40±0.02[b]	0.47±0.04[b]
3	Fructose isomer	1860	50.72±0.21[b]	61.07±0.19[a]	44.40±0.18[c]
4	Fructose isomer	1869	15.35±0.12[c]	31.78±0.08[a]	18.55±0.21[b]
5	Galactose isomer	1884	11.90±0.13[c]	16.77±0.14[a]	14.13±0.16[b]
6	Glucose isomer	1896	7.17±0.14[c]	14.73±0.17[a]	7.99±0.08[b]
7	Galactose isomer	1907	15.82±0.16[c]	24.60±0.12[a]	19.17±0.13[b]
8	Glucose isomer	1916	10.16±0.23[c]	12.54±0.16[a]	11.41±0.15[b]
9	Glucitol	1930	6.08±0.09[b]	6.89±0.14[a]	4.75±0.16[c]
10	Myo-Inositol	2090	2.61±0.12[c]	6.81±0.12[a]	5.99±0.09[b]
11	Sucrose isomer	2649	35.68±0.19[c]	51.58±0.17[a]	40.18±0.24[b]
12	Sucrose isomer	2660	11.83±0.13[c]	21.44±0.20[a]	14.44±0.16[b]
13	Turanose isomer	2742	26.46±0.18[c]	29.86±0.23[a]	27.65±0.18[b]
14	Turanose isomer	2766	21.82±0.24[c]	25.73±0.18[a]	23.50±0.13[b]
15	Arabinose isomer	1671	0.20±0.03[b]	0.29±0.01[b]	0.47±0.02[a]
16	Erythrose isomer	1470	1.31±0.08[a]	0.98±0.04[c]	1.10±0.04[b]
17	Erythrose isomer	1483	2.31±0.12[b]	2.01±0.06[c]	2.82±0.09[a]
18	Xylitol	1695	0.59±0.04[c]	0.97±0.06[a]	0.79±0.04[b]
19	Arabitol	1728	0.70±0.08[b]	0.53±0.04[c]	1.07±0.06[a]
20	Galactitol	1952	13.24±0.16[a]	12.24±0.17[b]	10.86±0.23[c]
21	Myo-Inositol-1-phosphate	2494	1.62±0.10[c]	9.10±0.11[a]	7.09±0.16[b]
22	Maltitol	2596	1.85±0.14[c]	8.44±0.16[a]	5.40±0.18[b]
	Fatty acids				
1	Palmitic acid	2040	4.18±0.15[b]	4.82±0.14[a]	0.84±0.04[c]
2	Stearic acid	2132	0.87±0.05[a]	0.45±0.03[b]	0.58±0.07[b]
3	Linoleic acid	2202	0.39±0.03[b]	0.50±0.05[a]	0.26±0.04[c]
4	α-Linolenic acid	2210	0.98±0.06[b]	1.32±0.07[a]	0.75±0.06[c]
	Others				
1	Phosphoric acid	1280	0.19±0.05[a,b]	0.28±0.03[a]	0.13±0.01[b]
2	Glycerol	1266	3.32±0.25[a]	2.49±0.09[b]	2.30±0.12[b]
5	Catechine	3220	4.27±0.16[b]	8.27±0.10[a]	3.73±0.08[c]
6	Stigmasterol	3315	1.43±0.10[b]	2.20±0.08[a]	1.08±0.04[c]
7	β-Sitosterol	3355	2.43±0.06[a]	2.41±0.07[a]	1.57±0.06[b]

RI – relative index (Kovats retention index);
*essiential amino acids;
[a,b,c] different letters in a row indicate significant differences (one way ANOVA, $p < 0.05$)

From the group of non-volatile polar metabolites, organic acids predominated, mainly quinic acid (47.50±0.12 μg.g^{-1} DW in L-SD22_Z extract, 33.28±0.19 μg.g^{-1} DW in L-UNTR22_Z extract and 26.11±0.15 μg.g^{-1} DW in L-SD22_M extract). In addition to these acids, low-molecular carbohydrates were detected in significant amounts – fructose isomer (61.07±0.19 μg.g^{-1} DW in L-SD22_Z extract; 50.72±0.21 μg.g^{-1} DW in L-UNTR22_Z extract; 44.40±0.18 μg.g^{-1} DW in L-SD22_M extract). The major aromatic constituents detected were linalool (17.29±0.21% of TIC for L-SD22_Z) and linalyl acetate (24.11±0.18% of TIC for L-SD22_Z). Linalool and linalyl acetate contribute significantly to the biological activity of the extracts and were found to inhibit the growth of some pathogenic microorganisms, such as *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. Moreover, linalool showed potent insecticidal, antitumor, anti-inflammatory, and antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*, and it is used in medicine, food industry and plant protection (Jirovetz et al. 2006). Oxidated sesquiterpenes were also found to be present in the highest amounts in the L-UNTR22 Z extract (46.05±0.15% of TIC). Due to the fact that the lavender extracts are rich in compounds with potent antibacterial activity the lavender residual biomass could be successfully applied as natural bio-preserving agents in the food industry (Vasileva et al. 2018). Six essential amino acids (L-valine, L-leucine, L-isoleucine, L-threonine, L-phenylalanine, L-lysine) were detected as the highest amount of them were found mainly in L-UNTR22_Z, while L-lysine and L-threonine were dominated in L-SD22_Z sample.

Four fatty acids were detected two saturated – palmitic C16:0 and stearic C18:0 acids and two unsaturated (linoleic acid C18:2 and α-linolenic acid (C18:3). In general, in all lavender samples palmitic acids dominated, while α-linolenic acid was in the highest values in L-SD22_Z sample.

Discussion

Of the three extracts, L-SD22_Z showed the highest mineral content: 7.45±0.20%. The degree of esterification was the highest in L-SD22_M: 83.9±0.5%, also in this extract the protein content was the highest: 8.15±1.6%. It was observed higher polyuronide content in L-SD22_Z sample: 8.0±0.2%. L-SD22_M extract had the highest polyphenolic content (total phenolics - 13.85±0.26 mg GAE.g^{-1} and total flavonoids - 1.16±0.02 mg QE.g^{-1}) and hence exhibited the highest antioxidant activity measured by: DPPH (51.70±0.40 mM TE.g^{-1}), ABTS (31.60±0.7 mM TE.g^{-1}), FRAP 84.27±22.76 mM TE.g^{-1}), and CUPRAC (385.27±4.78 mM TE.g^{-1}). The individual phenolic acids found in the extract were gallic, protocatechuic, syringic, p-coumaric, ferulic, rosmarinic, and salicylic acids. The highest amount was found for ferulic acid (298.06±0.59 μg.mL^{-1}) followed by rosmarinic acid (194.55±0.36 μg.mL^{-1}) in L-SD22_Z extract. Salicylic acid at 84.87±0.62 μg.mL^{-1}was found in L-SD22_Z extract and syringic acid (43.14±0.32 μg.mL^{-1}) in L-UNTR22_Z extract. The flavonoids found in the three extracts were (+)-catechin, (-)-epicatechin, rutin, hesperidin, quercetin. Sixty one compounds from groups of amino acid, organic acid, phenolic acid, low molecular carbohydrates, fatty acid, and other were detected in the extracts on subsequent analysis by GC-MS (Table 5). Polar volatile/aromatic compounds belonging to the hydrocarbon monoterpenes, oxydated monoterpenes, hydrocarbon sesquiterpenes, oxydated sesquiterpenes, and fatty acid esters were also identified (Table 6).

Confronting the results of the GC-MS analyses for content of polar volatile and non-volatile substances of the present study (2022 crop) with the results for crops 2020 and 2021 crops (Marovska et al. 2023) it could be concluded that from the amino acids L-proline was the dominating one for 2022 samples 2.56±0.16 – 2.92±0.16 μg.m^{-1}, while for 2020-2021 samples – L-threonine was found in the highest amounts (9.76±0.13 – 13.54±0.16 μg.mL^{-1}) followed by L-proline (2.03±0.09 – 2.82±0.07 μg.mL^{-1}) From the group of organic acids the most abundant one in the extracts from 2022 was quinic acid ranging from 26.11±0.15 μg.mL^{-1} to 47.50±0.12 μg.mL^{-1}. These results are observed also for the extracts for 2020-2021 samples – the highest concentrations (44.88±0.22 – 62.26±0.20 μg.mL^{-1}) were found also for quinic acid. In all the investigated extracts from 2022 (present study), 2021, 2020 (Marovska et al. 2023), and 2016 crops (Slavov et al. 2018) the most abundant compounds belonged to the low-molecular carbohydrates (glucose, fructose, galactose, xylose, arabinose, sucrose, turanose, etc.), and their derivatives – sugar alcohols.

Table 6. Polar volatile/aromatic compounds present in 70% ethanol extracts

№	Compound	RI	L-UNTR22_Z	L-SD22_Z	L-SD22_M
			Hydrocarbon monoterpenes		
1	α-Pinene	939	nd	0.16±0.02	nd
2	β-Myrcene	991	nd	0.37±0.03	nd
3	β-Pinene	979	nd	0.20±0.01	nd
4	p-Cymene	1019	nd	0.25±0.05	nd
5	Limonene	1025	nd	0.19±0.02	nd
6	(Z)-β-Ocimene	1039	nd	0.15±0.01	nd
7	(E)-β-Ocimene	1049	nd	0.23±0.02	nd
			Oxydated monoterpenes		
1	Eucalyptol	1031	nd	0.55±0.03	nd
2	cis-Linalool oxide	1073	nd	2.33±0.07	nd
3	trans-Linalool oxide	1078	nd	2.10±0.06	nd
4	Linalool	1097	0.43 ± 0.12^{b}	17.29 ± 0.21^{a}	0.51 ± 0.04^{b}
5	Lavandulol	1173	0.60 ± 0.05^{c}	0.90 ± 0.06^{a}	0.72 ± 0.02^{b}
6	Terpinene-4-ol	1177	0.35 ± 0.04^{b}	1.26 ± 0.04^{a}	0.42 ± 0.03^{b}
7	α-Terpineol	1189	0.19 ± 0.02^{b}	3.61 ± 0.09^{a}	0.23 ± 0.01^{b}
8	Geraniol	1253	nd	0.49±0.02	nd
9	Linalyl acetate	1255	0.10 ± 0.01^{b}	24.11 ± 0.1^{a}	0.12 ± 0.02^{b}
10	(±)-Lavandulyl acetate	1290	0.18 ± 0.02^{b}	2.73 ± 0.14^{a}	0.22 ± 0.01^{b}
11	Neryl acetate	1366	nd	2.30±0.08	nd
12	Geranyl acetate	1382	nd	6.30±0.16	nd
			Hydrocarbon Sesquiterpenes		
1	β-Caryophyllene	1415	0.49 ± 0.03^{b}	4.40 ± 0.18^{a}	0.59 ± 0.03^{b}
2	(E)-β-farnesene	1458	0.34 ± 0.02^{b}	3.45 ± 0.12^{a}	0.41 ± 0.02^{b}
3	Germacrene D	1464	10.93 ± 0.06^{a}	1.66 ± 0.04^{c}	8.12 ± 0.15^{b}
4	γ-Cadinene	1505	14.97 ± 0.09^{b}	3.93 ± 0.16^{c}	17.97 ± 0.08^{a}
			Oxydated sesquiterpenes		
1	Caryophyllene oxide	1580	46.05 ± 0.15^{a}	9.40 ± 0.21^{a}	40.25 ± 0.16^{b}
2	τ-Cadinol	1625	5.29 ± 0.16^{b}	2.48 ± 0.14^{c}	6.35 ± 0.08^{a}
3	τ-Muurolol	1630	9.50 ± 0.10^{b}	3.89 ± 0.10^{c}	11.42 ± 0.09^{a}
			Fatty acid esters		
1	Methyl palmitate	1920	3.23 ± 0.16^{b}	1.75 ± 0.11^{c}	3.90 ± 0.12^{a}
2	Methyl linoleate	2095	2.39 ± 0.10^{b}	0.72 ± 0.09^{c}	2.85 ± 0.10^{a}
3	Methyl oleate	2103	2.08 ± 0.08^{b}	1.30 ± 0.04^{c}	2.50 ± 0.09^{a}
4	Methyl octadecenoate	2124	2.26 ± 0.09^{b}	0.95 ± 0.07^{c}	2.72 ± 0.08^{a}

TIC – total ion current;
RI – relative index (Kovats retention index);
nd – not detected;
^{a,b,c} different letters in a row indicate significant differences (one way ANOVA, $p < 0.05$)

Comparing the aroma (volatile) substances found it could be concluded that for 2022 lavender samples in the highest concentrations from the oxydated monoterpenes were found linalyl acetate (24.11±0.18 μg.mL^{-1}), (17.29±0.21 μg.mL^{-1}), and geranyl acetate (6.30±0.16 μg.mL^{-1}). For the 2020 and 2021 crops the predominating oxydated monoterpene was again linalool but in much lower concentrations (from 3.15±0.04 to 7.89±0.08 μg.mL^{-1}). From the oxydated sesquiterpenes, caryophyllene oxide was found in higher concentrations in L-SD22_M (40.25±0.16 μg.mL^{-1}), while in the 2020 and 2021 lavender samples it was 10 times lower ranging from 3.41±0.04 to 5.55±0.09 μg.mL^{-1}. These results suggested that although the processed and the unprocessed biomass were collected from same locations for several years and the main polar volatile and non-volatile components are similar, some variations (significant and non-significant) in their concentrations exists.

Conclusions

In the current study three types of lavender: (L-SD22_Z), (L-SD22_M) after steam distillation and dried unprocessed lavender (L-UNTR22_Z) were compared. The L-SD22_Z sample was the richest of polar volatile/aromatic compounds (30 volatile compounds were detected), as linalool, (±)-lavandulyl acetate and (±)-lavandulyl acetate were in the highest amount. This lavender sample demonstrated the highest mineral (7.45±0.20%) and polyuronide (8.0±0.2%) contents, as well as some phenolic acids. The highest antioxidant potential and total phenols demonstrated L-SD22_M sample. It could be concluded that lavender residues obtained after industrial steam distillation are rich in biologically active substances, which suggested that the residual biomass could be used in the pharmaceutical, cosmetic and food industries.

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